Microbiological Deterioration of Organic Materials:

Its Prevention and Methods of Test

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UNITED STATES DEPARTMENT OF COMMERCE • Charles Sawyer, *Secretary*
NATIONAL BUREAU OF STANDARDS • E. U. Condon, *Director*

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by Edward Abrams

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PREFACE

The deterioration of textiles, leather, and other organic materials by mildew and rot is a major problem in the tropics, and is also important in many parts of the continental United States. Accordingly, it has seemed worth while to present the information on the subject that has been accumulated by the National Bureau of Standards in the past several years, and to describe the methods currently used to ascertain the susceptibility of materials to mildew growth and to evaluate protective treatments. Part I has been written primarily for persons looking for general information on the cause and prevention of mildew damage. Part II is addressed more particularly to the technologist and the laboratory worker.

E. U. Condon, *Director.*

Contents

Part 1. Review of the Literature

	Page
Preface	II
I. Introduction	1
II. Microbiology	1
III. Enzyme action	4
IV. Organic materials utilized by microorganisms	5
1. Cellulosic materials	8
2. Proteinaceous materials	10
3. Leather	10
4. Miscellaneous materials	10
(a) Rubber	10
(b) Plastics	11
V. Prevention of deterioration	11
VI. Testing for fungicidal efficiency	14

Part 2. Investigation of Microbiological Tests

VII. Development of mildew testing at Bureau	18
VIII. Representative problems	22
IX. Test methods	25
1. Pure-culture biological methods	25
(a) Rot-resistance tests with *Chaetomium globosum*	25
(1) Direct-inoculation—sterilized specimen method	25
(2) Mycelial mat method	26
(b) Mildew-resistance tests with *Aspergillus niger*	26
(1) Mycelial mat method	26
(2) Sugar-free medium method	26
2. Mixed-culture biological test methods	28
(a) Soil-burial test	28
(b) Soil-suspension test	28
3. Accelerated weathering test	28
X. Experimental results and discussion	29
XI. Summary and conclusions	38
XII. References	39

Microbiological Deterioration of Organic Materials: Its Prevention and Methods of Test

By Edward Abrams *

This paper presents a detailed review of the literature on the microbiological deterioration of organic and fibrous materials. Also, representative problems incidental to the development of test methods for the evaluation of mildew resistance and the development of mildew resistant materials are given. The most widely recognized test methods for the evaluation of mildew-resistance and rot-resistance are listed. A wide variety of fungicides for different uses is classified according to effectiveness. Plastics and plasticizers and related materials are rated according to their susceptibility to support mold growth.

Part 1. Review of the Literature

I. Introduction

The deterioration of organic materials by microorganisms in nature is of vital importance to man. Everyone is familiar with the accumulation of leaves and plant material in the fall. However, by the following spring practically all of this debris becomes a part of the soil. In this way nature renews her store of energy, the microorganisms completing the carbon, nitrogen, and sulfur cycles. Unfortunately, microorganisms are not discriminating in selecting their nutriment and are, therefore, a source of great loss when they attack organic materials of economic value.

From ancient times man has been plagued by mildew. As no method of control was known, it has been regarded as a necessary evil. A list of materials attacked by mildew would cover the entire field of organic materials. Because of ignorance and inertia a great waste of materials has been tolerated through the ages. Under favorable conditions of moisture and temperature many substances are attacked by molds. The present paper is restricted to the consideration of cellulosic and proteinaceous materials, rubber, and plastics. Foodstuffs are not included because their treatment presents an entirely different problem.

II. Microbiology

The terms *microorganisms, molds,* and *mildews* have been used interchangeably. Strictly speaking, in this field the term *microorganisms* should also include bacteria. However, this discussion is restricted to the effects of molds and mildews (the terms are synonymous) as the moisture level required for deterioration by bacteria is rarely encountered except in specialized fields, such as the maritime industry.

Molds are filamentous, branching forms of plant life. They belong to the subphylum Fungi of the phylum Thallophyta. According to definition they are plants that do not have roots, stems, or leaves. They are free of chlorophyll and require preformed organic materials and certain inorganic substances for their growth. Most molds are prolific spore producers. The spores serve to propagate the individual as do the seeds of higher plants. These spores are formed either at the tips of mycelial branches or are borne in special bodies known as sporangia or perithecia. They are liberated readily in vast numbers and float easily in the air. In this condition they are able to survive for long periods of time and under extreme conditions of humidity and temperature. Figure 1 shows the sporehead of *Aspergillus niger* magnified 1,000 times.

The number of genera and species of molds that have been isolated and classified runs into the thousands. They vary greatly in their morphology, physiology, and biochemical activities. Most of them are aerobic. They thrive at temperatures between 10° and 37° C and in the pH range from 2 to 8, with optimum growth at pH 4 to 7.

* Present address: Institute of Textile Technology, Charlottesville, Va.

FIGURE 1. *Spore head of Aspergillus niger.*

Many of the delicately attached spores can be seen floating in the field. Magnification, 1,090×.

Molds attack a great variety of organic compounds, including simple carbohydrates, fats, waxes, oils, cellulose, lignins, proteins, and even synthetic plastics and elastomers. In the process of growing they produce a variety of organic acids, including oxalic, citric, fumaric, gluconic, and carbonic. When protein materials are the source of nourishment they usually liberate ammonia. However, not all molds have the same capacity for attacking these different substances. Some prefer cellulosic materials, whereas others prefer protein materials. With regard to food utilization, molds are extremely efficient. It has been reported that as much as 50 to 60 percent of the decomposed substrate is converted to the cell substance, which includes the mycelium and the spores.

The soil is a rich source of molds. Each gram of soil contains between 10,000 and 200,000 spores and bits of mycelium. Dry soil, in the form of dust, carries the spores everywhere. When the temperature and humidity are favorable the

spores germinate and develop a branching mycelium. The food supply in the spore becomes exhausted rapidly, and the further development of the mycelium is controlled entirely by the amount of available nutrients in the substrate. In some cases, the mycelial threads of some of the molds can extend laterally for long distances away from their food supply over nonnutritive surfaces, such as glass or metal. This type of growth may result in surface etching due to the excretion of acids or alkalies by the mold.

Mold growth causes "rot" in the case of the cellulose destroyers, or "mildew" in the case of the surface growers. Rot is measured by loss in breaking strength, whereas mildew is measured by the visible amount of mold growth on a test specimen.

Holzinger [83] in 1909 studied the influence of osmotic processes in a medium on the growth of microorganisms. In 1910 Artari [9] continued work along the same line and studied the influence of the concentration of nutrient media on the growth of algae and fungi. The most rapid growth took place in 10 percent glucose solution, which he found to be the optimum concentration. Solutions prepared with less glucose but with other material sufficient to establish an osmotic pressure equivalent to that of the 10-percent solution did not support equally rapid growth.

Kellerman and McBeth [95] in 1912 reported that, contrary to Omeliansky's observation that cellulose-destroying bacteria do not grow upon solid media, most of the species isolated by them grew readily upon such media as beef agar, gelatin, starch, potato, and dextrose. Some, in fact, liquefy gelatin. Molds were found to be as effective as bacteria in destroying cellulose. They claimed to have isolated 75 species of molds representing a large number of genera. Species of *Penicillium*, *Aspergillus*, and *Fusarium* were most numerous. They pointed out that in the destruction of pure cellulose either by bacteria or molds in synthetic media, the associative action of organisms that presumably have no cellulolytic enzymes frequently stimulate the growth of the cellulolytic organism and increase its destructive power. This has been borne out by much recent work in which it has been shown that many organisms require special growth substances for proper development. These substances are normally obtained from other organisms in a more or less symbiotic relationship. To illustrate, the Australian workers in this field have proposed the use of an active cellulose destroyer, *Memnoniella echinata*, as a test organism. Although this organism is assuredly an excellent cellulose destroyer, it will not grow upon synthetic media unless it is contaminated by another organism or it is supplied with biotin. It is believed that the contaminating organism supplies biotin.

Scales [139] using McBeth's methods, continued the work of isolating cellulolytic organisms and isolated 30 species of *Penicillium* and 10 species of *Aspergillus*.

In 1917 Linossier [101] studied the growth characteristics of *Oidium lactis*. He found that the yield of *O. lactis* was distinctly proportional to the weight of the food. The exception to this was a group of mineral substances that seemed to act as catalysts, exerting their maximum action at minimum dose. However, when food was given in increasingly greater amounts than could be assimilated during the time of the experiment, the yield increased less and less, finally reaching a maximum. Increasing the food concentration beyond this point a harmful effect on the yield was noted, especially with reference to nitrogenous food.

Roberg [134] in 1928 studied the growth characteristics of *Aspergillus niger*, *A. fumigatus*, and *A. oryzae*. He found that these organisms would not grow in the absence of Fe or Zn. In the absence of Cu there was mycelial growth but little fruiting. Also, in the absence of Cu, *A niger* did not become black.

Galloway [64, 65] in 1930 and 1931 isolated molds from mildewed cotton fabrics and found that they were mainly *Aspergilli*, *Penicillia*, and *Fusaria*. He attempted to explain the cause of what was commonly called "diamond spot" stains. These he believed were caused by the growth of organisms that would begin at the intersection of two fibers and spread from this point.

Karnicka and Ziemiecka [91] in 1935 reported that molds were the principal agents of cellulose decomposition in soils with a low humus content, especially when the pH was less than 6. Fertilization with nitrogen stimulated fungal activity, whereas applications of lime diminished it. Thirteen molds, including four of the genus *Chaetomium*, were isolated from an acid soil. Their study revealed that in acid soils cellulose is first attacked by fungi, followed by actinomycetes, and finally by bacteria. In neutral soils bacteria start the degradation.

In further studies Galloway [66] in 1935 showed that the rate of spore germination is governed primarily by the atmospheric humidity, rather than the moisture content of the substrate. Considerable variation is shown in the moisture requirements of the more common groups of molds. The minimum relative humidity varies from 75 to 95 percent. *Aspergillus glaucus*, *A. candidus*, and *A. versicolor* can develop at 75- to 80-percent relative humidity.

Molds have been found capable of growing under extreme conditions of temperature and pH. Thom [164, 165] cites evidence of mold growth at low temperatures, as in refrigerators. He reports

the presence of molds in nickel electroplating solutions. Porges [129] in 1938, reported a fungus growth in a copper-plating bath containing a saturated solution of $CuSO_4$ and 6.8 percent of H_2SO_4.

Mulder [115] in 1939 verified Roberg's [134] findings with regard to the necessity of copper for the growth of *Aspergillus niger*. He found that the presence of cadmium increases the copper requirement. Many other species of *Aspergillus* and also *Acetobacter aceti* also require copper for growth. Mulder suggests that *A. niger* may well be used to estimate the copper content of soils.

Corroborating the work of Karnicka and Ziemiecka [91], Skinner and Mellem [152] in 1944 added finely divided filter paper to acid soils that were 60 percent saturated with water. They found no evidence of the activity of cellulolytic bacteria, although mold growth increased greatly in spite of the excessive moisture. In soils with a pH above 5.0, both molds and bacteria showed an increase. The conclusion of Dubos [51] that both aerobic bacteria and molds take part in the decomposition of cellulose in nonsaturated soils, unless they are distinctly acid, is shown to be correct.

In a study of a wide variety of molds, with the object of finding a more suitable cellulolytic test organism, Pope [128] in 1944, working with Greathouse, isolated and identified a new organism, *Metarrhizium glutinosum*, from a bale of deteriorated cotton. It was found to have extraordinary cellulolytic power and was used in the evaluation of mildew-proofing agents. White and Downing [172] in 1947 have shown this organism to be *Myrothecium verrucaria*.

Zuck and Diehl [179] in 1946 found that certain angiocarpous fungi, which had heretofore been unrecognized as cellulose destroyers, were significant agents in the slow cellulosic breakdown of fabrics during aerial exposure. Among these organisms are *Diplodiella cowdellii*, *Hendersonia sarmentorum*, *Leptosphaeria sp.*, *Diplodia sp.*, *Phoma herbarum*, and *Ophiobolus sp.* These fungi have tended to escape detection because of the employment of standard plating techniques that do not support the growth of these slow-growing fungi.

The retting of bast fibers, such as jute, flax, and ramie, is also a form of microbiological deterioration. Here, however, man has been able to derive some benefit from the activities of these organisms. Formerly [93] it was believed that the process of retting was merely the breakdown of pectin and other cementing substances with the ultimate separation of individual fibers. It was not believed that these organisms could be responsible for cellulolytic activity. As the process had developed from ancient times, all information relating to it was of a more or less empirical nature. However, by 1940 Ruschmann and Bartram [138] were able to show that the best quality of bast fibers are obtained when the conditions of retting are carefully controlled. They found that the aerobic organism *Alternaria tenuis* destroys both pectin and cellulose rapidly. If the process is permitted to go to completion, the bast fiber is completely destroyed.

III. Enzyme Action

No discussion of biological activity can be complete without investigating the role played by enzymes. To understand the role of enzymes in cellulose deterioration a knowledge of cellulose is required. The molecule of pure cellulose is composed of beta-glucose units linked through the 1–4 positions to form long chains. Before it can be utilized by microorganisms as a source of energy it must be broken down to water soluble molecules. This degradation, previous to oxidation by the cell respiratory system, is enzymotic, probably mainly hydrolytie, and is believed to be the work of more than one enzyme.

De Bary [47] in 1886 was the first to make a careful study of the dissolution of cell walls by plant fungi. The formation by these organisms of a cellulose-dissolving enzyme, later designated as cellulase, was thus indicated. In 1888 Ward [171] reported, from microscopic observations, that the fungus, *Botrytis sp.*, secreted an enzyme capable of hydrolyzing the cell walls of the host tissue. Von Euler [55] obtained from the wood-destroying fungus *Merulius lacrymans* an enzyme that acted upon the hydrolytic products of cellulose but not on cellulose itself. Pringsheim [130] in 1912 reported a cellulase from thermophilic cellulose bacteria. The decomposition of cellulose by these bacteria was believed to occur in two stages: (1) the reduction of cellulose to cellobiose, and (2) cellobiose to glucose. The enzymes were considered to be endoenzymes as the cell-free extracts had no action on cellulose. Schmitz [140], in 1920, obtained cellulase from a great number of molds. Karrer, et al. [92], in 1923, showed that cellulase is present in the digestive system of the edible snail. Selliere [144, 145, 146], from 1905 to 1910, examined the hydrolysis of cellulosic materials by enzyme action, the end product being glucose. He found that cellulose was attacked more rapidly if it was first treated with 25 percent caustic soda or with $ZnCl_2$ or precipitated from Schweitzer's reagent. He concluded that the physical condition of the cellulose was an important factor in determining the rate of hydrolysis under enzyme

action. Grassmann and Rubenbauer [72] in 1931 stated that a dialysed and concentrated enzyme extract from *Aspergillus oryzae* hydrolyzed filter and parchment papers at a slow rate. Grassmann, et al. [73] in 1933 found that a crude extract of this fungus hydrolyzed many polysaccharides. It hydrolyzed a cellulose prepared from beechwood at a slow rate but a hydrocellulose prepared from the same source and one prepared from cotton was hydrolyzed 25 times as fast. The optimum pH was 4.5. Clayson [41] in 1942 found that the ease of decomposition of naturally occurring cellulosic materials appears to be determined largely by whether the encrusting substances are readily attacked or are resistant. Schonleber [141] believed that the orientation of rows of micelles parallel to the longitudinal axis of rayon fibers gives the fibers greatly increased resistance against the penetration of cellulose-dissolving enzymes.

Various workers have claimed to separate cellulase and cellobiase. Either these are different substances (pH optima varied from 5.9 to 4.7), or they are influenced by contaminants. According to Norman and Fuller [120], in 1942, the existence of an hydrolytic enzyme system capable of producing either glucose or cellobiose from cellulose may be considered proved but what is lacking is any information of the intermediary steps. In view of the fact that growth of some cellulolytic organisms is inhibited by low concentrations of glucose, it may well be that the disintegration does not normally proceed through glucose.

Depending on concentration, acids, bases, and salts will either stimulate or inhibit enzyme action. The concentration of salts of heavy metals necessary to inhibit enzyme action depends on the nature of the enzyme and the metal, according to Hata [81] in 1904. The activity of many catalase preparations is reduced 50 percent by 0.005 mg of $HgCl_2$ per liter. However, Kehoe [94] in 1922 stated that high concentrations of certain neutral salts of the alkalies and alkaline earths will reactivate enzymes inactivated by the salts of heavy metals. Robbins [133] in 1916 found that salts not only affect the activity of enzymes but also their formation by microorganisms. Some organic chemicals also poison enzymes. Among these are HCN, HCHO, alcohols, and chloroform. Light may either destroy or inhibit enzymes especially in the presence of oxygen and fluorescent substances.

Ziese [176] in 1931 worked with cellulases from various sources and found them to be relatively thermostable. In a dilution of 1 to 2,000, they may be heated 5 to 10 minutes at 100° C. without complete loss of activity. A 1 percent solution of $CuSO_4$ and 3 percent of HCN separately have practically no effect, but when mixed they are strongly inhibitory. Glutathione and cysteine, but not H_2S or $Na_2S_2O_3$, are inhibitory when phosphate buffer is used, but not when citrate buffer of the same pH is used. The complexity of this subject may be a result of the fact that microorganisms that attack cellulose may be separated into three groups, depending on oxygen intake, according to Dubos [51]: (1) strictly aerobic forms which are specific to cellulose, (2) strictly aerobic forms which attack both cellulose and starch, and (3) facultative anaerobes which attack cellulose.

In 1945 Siu and White [151] reexamined the evidence for enzymotic break-down of cellulose. They decided that the enzymotic break-down of cellulose is an often quoted but unconfirmed theory. The theory assumes the presence of two enzymes. Cellulase is believed to hydrolyze cellulose to cellobiose and then cellobiase converts cellobiose into glucose. They found that claims have been made that these two enzymes have been separated by the selective adsorption of cellobiase on alumina. The crude cellobiase thus obtained presumably is capable of hydrolyzing oligosaccharides of six anhydroglucose units or less, while the crude cellulase preparation can attack only those of greater complexity. In reviewing previous work in this field they found that most of the preliminary work was done with crude enzymotic preparations from the digestive tract of the edible snail, *Helix pomatia*, and the ground mycelium of *Aspergillus oryzae*. Ground filter paper or wood was exposed to attack by the preparation in question, and the increasing reducing ability of the resulting mixture was used as the index of enzymotic action. They conclude by saying that despite all of these studies, up to the present time there has been no confirmed isolation of a cell-free preparation capable of hydrolyzing cellulose. They found no cellulolytic activity in the metabolic filtrate of *Cytophaga D* or *Myrothecium verrucaria* (*Metarrhizium glutinosum*) concentrated 30 times.

IV. Organic Materials Utilized by Microorganisms

1. Cellulosic Materials

Among the materials which have been reported as being subjected to microbial degradation, cotton and other cellulosic materials rank highest. Figure 2 shows cotton fabric heavily invaded by *Aspergillus niger*. The earliest reports in this field are, more or less, observations of mold activity. In 1912 Osborn [125] reported that mildew of cotton cloth appears to be due to many common saprophytic molds which, he believed, fed upon the starchy matter in the sizing. He

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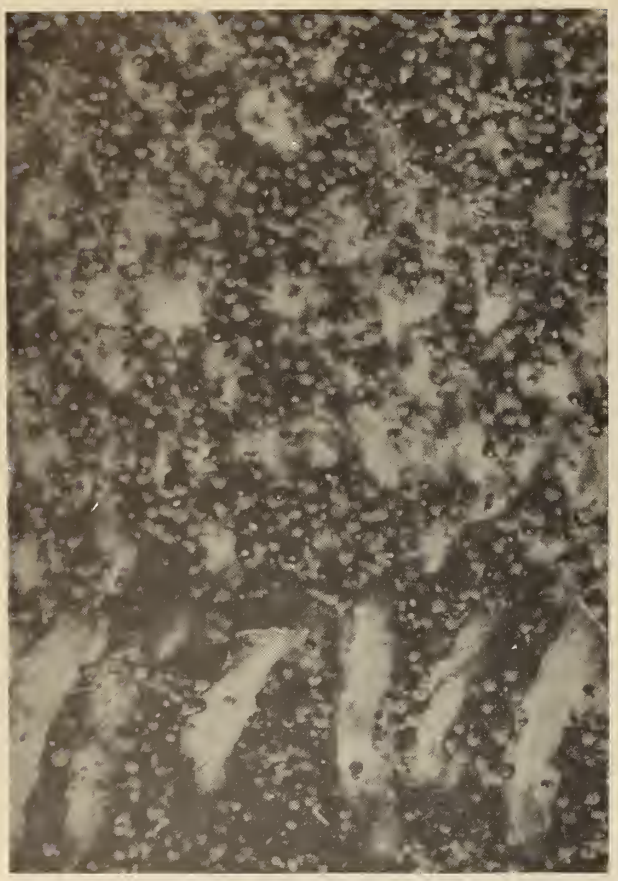

FIGURE 2. *Camouflage fabric with inferior fungicide showing the invasion of the weave by the mycelium and spore-heads of Aspergillus niger.*

pointed out that the fungi could grow only in the presence of sufficient moisture. Therefore, if cotton materials were not permitted to become damp, they were not likely to become moldy. In 1913 Kroulik [97] reported that microorganisms, which he believed to be bacteria and actinomycetes, decompose cellulose even at temperatures as high as 60° to 65° C. In the same year, McBeth and Scales [111], unlike the other workers of the period, were actually working at the causes of cellulose degradation by microorganisms. They found that a selective medium like cellulose agar was superior to Omeliansky's selective culture method [100], and the filter-paper method of Van Iterson [100]. They found that molds and bacteria, capable of destroying cellulose, are present in all productive soils. The cellulose-destroying types attack cellulose most rapidly in the presence of oxygen and often lose their power to do so when grown on artificial media. The truth of this fact accounts, in part, for the great difficulty in finding suitable test organisms that will maintain their growth characteristics from generation to generation over long periods of time. In the same year, Mutterlein [116] found that aerobic organisms were more important than anaerobes in the decomposition of cellulose in nature. In 1915, Guegen [78] reported that the black spots and patches that tend to develop on tent cloth and sails when exposed to damp air, and that cause weakening of the fibers as if by sulfuric acid, are due to the growth of molds. He listed two organisms as being especially destructive—*Pleospora infectoria* and *P. herbarum*. He believed that the spores of these molds were rarely airborne but were in the tissues of the textile fiber.

In 1916, Otto [127] made as thorough a study of the cellulolytic activity of microorganisms as had been made up to that time. He used a wide variety of cellulosic materials as substrates including blotting paper (true cellulose), various natural celluloses, and modified celluloses such as oxycellulose, hydrocellulose and hydrated cellulose. He found that the response of molds to these substrates varied considerably. But he was able to report that soil molds were capable of "dissolving" cellulose. He believed that "solution" of cellulose was effected by means of enzymes which caused hydrolytic cleavage. The cleavage of various types of cellulose was effected by the same molds, with hydrolytic cleavage taking place in those molecular groups which were common to all the forms of cellulose tested.

Hutchinson and Clayton [85] in 1919 isolated a new cellulose-destroying bacteria, *Spirochaeta cytophaga*, which was to be studied later by Thom and his coworkers for possible use as a test organism. Hutchinson and Clayton studied the growth habits of this organism and reported that it was aerobic and had great cellulolytic power.

For the next few years various authors were reporting in the same vein. This was the period following the First World War, and it is probable that as a result of the war much practical information had been gathered. Armstead and Harland [4, 5], Fleming and Thaysen [58, 59], and Thaysen and Bunker [158] reported on the effects of microorganisms on textile fibers, especially on the fact that cottons of different origins had varying resistance to destruction by microorganisms. By 1924 Thaysen [159] was able to sum up his observations with the following: Moist cellulose-containing materials, when not incrusted with lignin, constitute a very suitable food for many types of microorganisms. In the presence of about 10 percent of moisture the spores develop a mycelium that may be entirely on the surface of the fiber, perforate it in places, or may even develop within the fiber. Certain bacilli also develop colonies that gradually penetrate the fiber. As these spores are normally present on raw cotton, the chances of infection in finishing processes are great. These organisms produce both chemical and physical or morphological

changes in the fiber, involving loss of strength and usually a marked discoloration. The penetration of the fiber is aided by the enzyme, cytase, which converts the cellulose into glucose. The glucose then is used by the organisms as a source of energy and on oxidation yields CO_2, H, and organic acids. A primary stage in the production of glucose is the hydration of the cellulose, wherein the fiber is more readily stained by dyes and retains the color better. The destructive action of the microorganisms on cotton may be followed, almost from the start, by the swelling action of a solution containing equal parts of CS_2 and 9 percent of NaOH. This reagent gives the normal fiber a microscopic appearance similar to that obtained with Schweitzer's reagent, that is, swelling with constrictions. Fibers with microbiological damage swell more quickly and present an almost uniformly swollen appearance without constrictions, possibly because of the destruction of the cuticle of the fiber. He confirms the findings of others by stating that acetylated cellulose is resistant to bacterial attack and that bleached cotton is more resistant than unbleached cotton. Finally, he agrees that some types of cotton are more resistant to degradation than others. American cotton is most resistant, whereas Indian cotton is least resistant. In 1925 Thaysen and Bunker [160] reported further on their work with various cellulose derivatives. They found that cellulose acetate was most resistant to microorganisms, whereas cuprammonium rayon was least resistant.

In 1927 Serrano [147] showed that the deterioration of abaca fibers (manila hemp) was due to the action of cellulose-digesting organisms of the genera *Aspergillus, Penicillium, and Chaetomium.* The growth of these organisms, he found, was favored by dampness, poor ventilation and elevated temperatures. Winogradsky in 1929 [174] made a careful study of cellulose decomposition and reported that cellulose undergoes rapid oxidation in the soil and the product closely resembles oxycellulose. The biological oxidation of cellulose, he said, is differentiated from the purely chemical process in that the product does not reduce Fehling's solution. Assimilable nitrogen, preferably inorganic, is essential to this process, the consumption amounting to approximately 2 parts of nitrogen for each 100 parts of cellulose decomposed. The consumed nitrogen is transformed into organic combination, but there is evidence of some partial reduction to ammonia even with unrestricted access to the air. The optimum reaction for cellulose decomposition is pH7. Slight changes of reaction occur during the process, depending on the form in which the nitrogen is supplied.

In 1930 Thaysen and Bunker [162] were the first to report on controlled field exposures in which cotton, wool, hemp, and flax fabrics were exposed to the action of microorganisms at Ceylon Cyprus, Kenya, Trinidad, the Malay States, and England. The materials were hung in the air, both in and out of sunlight; buried in the soil; and submerged in the sea at Ceylon. In marine waters disintegration was most rapid, and was mainly the result of bacterial action. In soils the deterioration was caused by bacteria, fungi, and termites. In the air the damage was produced by molds and actinomycetes. They found that hemp and flax were most resistant to degradation.

Up to 1937 the most thorough study of cellulose destruction in the soil was made by Madhok [107]. He found that the most rapid decomposition of cellulose in the soil took place at an initial pH of 5.5 with $NaNO_3$ as a source of nitrogen. This indicated that the first rapid attack was by fungi, followed later by bacteria as the reaction tended toward neutrality. Decomposition started most rapidly at 37° C, but later the rate at 27° and 30° C approached the 37° C rate. Fertilization of the soil over a period of 25 years had no appreciable effect on the microorganisms decomposing cellulose. This latter observation was later verified by Batson, Teunisson, and Porges [16.] In the same year Galloway [67, 68] published a general review of the microbiology of textiles. He pointed out that the microbes were useful as well as harmful, as mold enzyme products were used in many textile procedures.

In 1938 Barker [12] made a study of various bast fibers, including flax, ramie, hemp, pita grass, jute, and sisal. In such fibers other polysaccharides are closely associated with cellulose and are conconsidered to be an integral part of the cellulose aggregate. Xylan is the most common of the carbohydrates retained by the fiber. The molecular structures of cellulose and xylan are similar, and the action of fungi is to hydrolyze the latter, breaking down its molecular chains into shorter lengths and thus causing loss of strength or rotting. He emphasizes the accelerating effect of moisture on the deterioration of fibers by microorganisms. In another paper [13] in the same year, Barker considers the use of antiseptics for the protection of cellulosic fabrics. He finds salicylanilide to be excellent for this purpose. The effectiveness of the Thaysen acetylation process [159] is again affirmed. He believes that it renders a cotton surface unsuitable as a nutrient source for microorganisms.

Shults [150] in 1938 reported that the development of *Aspergillus niger* can be inhibited by drying cotton fabrics to 20-percent moisture content followed by storage at 71 to 73-percent relative humidity. This is at variance with Thaysen and Bunker [158], who claim that 10 percent of moisture is sufficient to cause germination of spores. Shults points out that viscose products are easily degraded by microorganisms. In 1939 Galloway [69] found that the minimum

moisture regain for the development of micro-organisms on jute fibers was 17 percent.

At about this time the trend in this field was toward the study of individual organisms that could be demonstrated to grow on a cellulose-containing medium. In 1939 Hooper [84] isolated from the soil an aerobic micro-organism that was capable of growing on an inorganic medium, with such cellulosic materials as filter paper or cotton as the sole source of carbon. Although the organism was studied in detail, it was not identified. By following the disintegration of cotton fibers under the microscope, Hooper found that the cuticle is removed from the fiber during the early stages of attack, and then both the cementing material and cellulose are slowly digested. The particulate nature of the cellulose fibrils of the cotton-fiber cell wall is clearly visible in the partially disintegrated fibers.

With the outbreak of war in Europe in 1939, the tempo of research in this field increased. Thaysen, et al. [163] found that in microbiologically active soil cellulose acetate rayon was completely resistant whereas cellulose, wool, silk, and cellulose rayon fabrics were disintegrated. Bryson [30] pointed out the need for preserving jute fabrics for sandbags. He believed the naphthenic acid derivatives and particularly copper naphthenate to be most promising.

In 1940 Waksman [170] recognizing the need for up-to-date information on cellulose deterioration published a review of the microbiology of cellulose decomposition. He pointed out that while cellulose was resistant to ordinary chemical reagents and the digestive juices of higher animals, it was readily decomposed by a great variety of micro-organisms.

In 1941 Armstrong [6, 7, 8] and Barker [14] studied the problems of jute-sandbag preservation. They found that damp jute bags were subject to rotting. Molds and bacteria of soils are responsible for this degradation. Molds attack jute that contains at least 17 percent of moisture, while at least 22 percent of moisture is required for attack by some strains of bacteria. Armstrong recommends a variety of preservative treatments and points out that treated jute bags can be expected to have a useful life of 18 months to 2 years, whereas untreated bags usually last a few weeks at best.

In 1945 Larnach and Wyke [98] investigated the problem of mold growth on books and paper. They observed that there was no growth of fungi when the relative humidity was below 75 percent. Therefore, to control mold growth they recommended the use of silica gel in book cases. In the same year Fargher [57] made a study of the growth substances present in cotton. He found that raw cottons contain appreciable quantities of K, Na, Ca, and Mg, all of which are essential to vigorous growth of molds. The principal trace metals, Fe, Cu, and Zn, which stimulate the growth of certain organisms are also present. Most of the metals are present as water-soluble salts of organic acids, which are consumed readily by micro-organisms. Also present are sulfate, chloride, phosphate, glucose, and more complex carbohydrates, and nitrogenous substances. Flour used in sizing affords an additional source of nitrogen as well as of carbon, and supports more prolific and rapid mold growth than starch. Modified starches are utilized more readily than are unmodified starches. The consumption of the naturally occurring food materials can be followed by measurements of reducing power (copper number) and pH of the cotton during exposure to damp conditions. When the water-soluble constituents are removed, cotton becomes more resistant to micro-organisms. The resistance becomes still greater after scouring with alkalies. For this reason it is believed that linen appears to be more resistant than cotton to microbiological degradation.

Bayley and Weatherburn [17, 18] in 1945 draw attention to the fact that while copper preservatives appear to be excellent in laboratory tests, they have been able to isolate copper-tolerant fungi from copper-treated fabrics.

Goldthwait, et al. [70] in 1946 reported that cotton fibers, yarns, or even fabrics could be partially acetylated in such a manner that the cotton retains its outward appearance but acquires several new properties. The resulting modified cotton is highly resistant to mildewing and rotting and has a lower moisture regain.

2. Proteinaceous Materials

Although the documentation of the microbiological degradation of proteinaceous materials is not as extensive as that for cellulosic materials, the problem is equally serious. The organisms involved are, in general, not the same as those which degrade cellulose [60, 153]. Organisms that attack proteinaceous materials may cause rotting of the material, as in the case of wool, or they may attack important substances in the protein material, as the oils in leather. Figure 3 shows a circle of vegetable-tanned leather, heavily overgrown by molds.

In 1924 Burgess [32] published a study of the types of molds and bacteria found on damaged wool. He believed that molds caused discoloration, whereas bacteria caused rotting. In 1926 Hayes and Holden [82] made the same observations with regard to silk. In further studies on wool, reported in 1928 and 1929, Burgess [33, 34] found that mildew on stored woolen goods was entirely due to molds rather than to bacteria. Soaps and other conditioners enhance its development. The alkalies combine with the wool render-

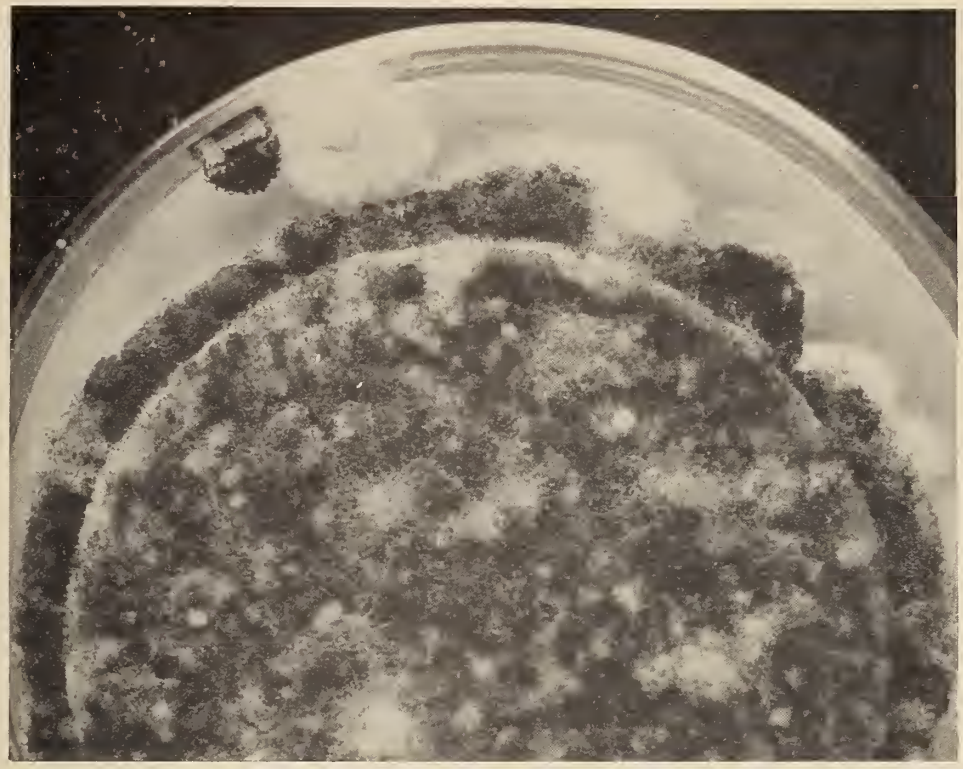

FIGURE 3. *Untreated vegetable-tanned leather on a sucrose agar plate uninoculated and incubated for 7 days a 28° C.* The growth, which completely covers the leather, is mainly *Aspergillus niger*. The ease with which leather supports a growth of mildew is thus demonstrated

ing it more easily attacked. Finishing materials that are hygroscopic in nature raise the moisture content to the optimum level for mold growth.

Thaysen and Bunker [162] in 1930 exposed woolen fabrics to the action of microorganisms at Ceylon, Cyprus, Kenya, Trinidad, the Malay States, and England. The fabrics were hung in the air, both in and out of sunlight; buried in the soil; and submerged in the sea at Ceylon. Except in air exposure the wool was rapidly degraded, especially by bacteria where moisture was relatively high.

Burgess [35] in 1930 reported that wool could be made mold-resistant by the use of chrome in the dye bath, and in 1934 [36, 37] that the amount of nutrient substances in wool greatly influences its mildew susceptibility, especially in a damp atmosphere. He noted that intense chlorination and treatment with hydrogen peroxide or ultraviolet light increases susceptibility to attack by molds and bacteria and that bacterial development requires actual wetness. At this time he recommended the use of salicylanilide as a preservative. Finally, in 1934, in an effort to study the mechanism of bacterial degradation of wool, Burgess [38] found that wool fibers could be disintegrated into their component elements of epithelial scales and cortical cells by treatment with an appropriately buffered solution of trypsin or pepsin. The action of the trypsin solution is similar to that of proteolytic bacteria, but is more rapid. He suggested this treatment as a laboratory method for determining the resistance of wool and other animal fibers to bacterial action.

In 1939 Thaysen, et al. [163] reported that wool and silk were completely disintegrated in microbiologically active soil. Harold [80] in 1941 pointed out that damage due to molds is of greater magnitude than that due to bacteria because of the greater amount of moisture necessary for bacterial growth. Wool in the grease or wool treated with oil emulsion tends to mildew at lower temperatures than scoured wools.

Schonleber [141] in 1941 found that casein fibers are more easily deteriorated by bacteria than by molds. She found two organisms to be especially active in this respect, namely, *Proteus vulgaris* and *Micrococcus sp.*

In 1943 Ayres and Tobie [10] found that molds and bacteria that have the power to hydrolyze proteins usually give very active preparations of proteases on appropriate treatment.

The paper industry, which requires large quantities of wool felt for use in paper-making machines, has been put to great expense by the short life of these materials due to the rapid rotting of the felts. The conditions are severe as these felts are completely wet for long periods of time. There

are many papers dealing with this subject but few workers have been able to solve the problem. Binns [24] in 1934 measured the extent of bacterial degradation of woolen felts by the loss in strength. He found that attack by bacteria depends in part on the source of the water used in paper manufacture. It is generally increased by the presence of phosphates and is inhibited by acid conditions. Binns suggested the use of various antiseptics but expressed the opinion that comparatively large concentrations are needed for protection.

3. Leather

Although leather is a protein substance, its degradation by microorganisms is quite different from that of other proteinaceous materials. Although many reports have been received from tropical battlefields to the effect that leather rots and falls apart rather rapidly, none of the specimens that were received were observed to be in this condition. Furthermore, it has not been possible to rot leather in the laboratory, even in soil-burial beds and in tropical room exposure tests. To be sure, leather articles such as shoes and equipment cases have been observed to fall apart, but on examination it was found that the stitching, usually cellulosic in nature, had rotted. No rotting was ever observed when nylon thread was used for stitching leather. As shall be shown, the primary damage to leather by microorganisms is the stiffening and shrinking caused by the removal of the oils and greases. Figure 4 shows

FIGURE 4. *Two cases of Army shoes received from storage on the island of Saipan illustrate the importance of packaging.*

The package on the right was in a badly deteriorated state when received, whereas the package on the left was in excellent condition. On opening the cases, the shoes in the package on the right were heavily mildewed.

mildew growth on new Army shoes stored under extreme conditions of temperature and humidity.

In 1925 Wilson and Daub [173] reported that after hides are tanned the greatest damage is caused by molds. Untanned hides, on the other hand, are easy prey for bacteria.

In regard to the fats and greases commonly found in processed leather, Vaubel [167] in 1928 found that molds attack free fatty acids to a greater extent than glycerides. Unsaturated fatty acids are more readily utilized than saturated acids and the *cis* form more than the *trans* form. Thus, oleic acid is more susceptible to mold growth than elaidic acid. In 1932 Grimshaw [77] found that both olive and lard oils are good sources of food for molds but that mineral oil is not as good a source.

With reference to the effects of mold growth on leather, Lloyd [102] in 1934 observed that molds may produce stains, destroy tannins, hydrolyze fat, produce spues, and under certain conditions may pit the surface.

A study of microbial growth on 55 leathers by Colin-Russ [43] in 1940 showed damage when the moisture content was about 30 to 35 percent of the total weight.

In 1945 Dempsey [48] found that chrome-tanned leather does not mold readily. Semichrome leathers mold more easily, whereas vegetable-tanned leather is most susceptible to mold growth. The direct effects of mold growth include damage of the grain, formation of stains that cannot be removed without damaging the grain, and, finally, destruction of pyrogallol tannins. Threads and fabric linings in leather articles may be affected by the mold growth on the leather. Free acid resulting from hydrolysis of triglycerides by molds may corrode metal fittings.

Kanagy, Charles, Abrams, and Tener [90] in 1946 made a study of the effect of mold growth on vegetable-tanned leather. They found that the growth of mildew on leather increases its stiffness, decreases its tensile strength and stretch at the breaking point, and weakens the grain surface. Chemical tests revealed a loss of grease, water-soluble materials, glucose, tannins, and nontannins. No appreciable deterioraton of hide substance was found. It was concluded that the molds, in assimilating the grease, bring about decarboxylation of the fatty acids, as shown by decrease in saponification number.

4. Miscellaneous Materials

(a) Rubber

The deterioration of rubber in the latex stage has been well documented and has been the subject of study for many years. However, the microbiological deterioration of cured rubber has not received equal attention.

In 1914 Sohngen [155] showed that microorganisms do not grow on air-dried rubber because of

the low water content, which he found to be approximately 0.5 percent. However, in the presence of large amounts of water, common India rubber furnishes a medium for the growth of bacteria and molds, inasmuch as it contains proteins, sugars, and resins.

Scott [142] in 1920 also showed that vulcanized rubber, especially pure gum compounds, develops growths of *Stemphylium macrosporoideum* when exposed to moist air. This growth was described as resembling dust. Some threads were seen to penetrate the surface and were probably responsible for the loss in strength that occurred.

In 1938 Kalinenko [89] dispersed rubber in a flask and inoculated it with *Aspergillus oryzae*. In 1 month he found that 32 percent of the rubber had been destroyed.

ZoBell and Grant [177] in 1942 studied the degradation characteristics of various types of rubber. Their criterion of rubber degradation was increased oxygen consumption and CO_2 production. They used inocula from sea water, garden soil, old garden hose, and cracks in tires. All of these inocula increased the oxygen consumption 5 to 10 times. The rate of oxygen consumption increased exponentially with time.

Dimond and Horsfall [49] in 1943 pointed out that wherever rubber is to be used either in contact with water or under conditions of high moisture, as in the tropics, a mildew-proofing agent should be used.

(b) Plastics

Plastic materials are of such recent origin that aside from the experience gained in the recent war, there has been no documentation of microbiological deterioration. On the contrary, for a time it was believed that because these materials were man made or synthetic they could not possibly be the source of microbiological nutrition. This fallacy was disproved early in the recent war, when it was found that in the tropics many types of plastics supported copious growths of microorganisms. Brown [29] in 1946 sums up a good portion of the information gained during the war by reporting the work of several laboratories.

V. Prevention of Deterioration

The early use of mildew-proofing agents was haphazard and was based on empirical rather than on experimental information. Fargher [57] in 1945 stated that prior to 1926, $ZnCl_2$, ordinarily in a concentration of 0.8 percent, was the principal mildew-proofing agent for fabrics but that it was not entirely effective. Other agents used were salicylic acid, sodium fluoride, sodium silicofluoride, phenol, cresylic acid, or formaldehyde.

Corrigan [44] in 1920 recommended the use of heat, light, and such antiseptics as copper sulfate, potassium nitrate, zinc sulfate, zinc chloride, boric acid, formaldehyde, and phenols for the destruction of fungal growths on finished goods.

In 1924 Thaysen [159] reported that acetylated cellulose was resistant to fungal attack. It was found that bleached cotton was more resistant to decay than unbleached cotton.

O'Brien [121, 122], working with rubber, reported in 1926 that a 0.1 percent solution of paranitrophenol was an effective mildew-proofing agent. In the same year Stevens [156], working with crepe rubber, also found paranitrophenol to be an effective fungicide.

The earlier observations that bright light tends to inhibit fungal growth and that ultraviolet light is actually fungicidal was explained by Bedford [22] in 1927, who found that destruction of microorganisms by ultraviolet light is due to the production of peroxide and is directly proportional to the quantity generated.

In 1928 a group of workers at the Shirley Institute in Leeds, Great Britain, investigated a large number of compounds for possible use as mildew-proofing agents. They found that salicylanilide was the most efficient of all compounds examined. In honor of the Shirley Institute they named it Shirlan. Fargher [57] in 1945 describes Shirlan as having all the desired properties:

1. Lack of volatility during fabric processing.
2. High degree of solubility to insure uniform distribution in fabric.
3. It is unaffected by heat, metal surfaces, or by sizing and other finishing materials.
4. It is colorless and odorless.
5. It has no undesirable action on textiles.
6. No undesirable action on subsequent bleaching or dyeing.
7. Low cost.
8. Harmless to man.

By 1930 Shirlan had been used rather widely, and Hall [79] recommended its use in place of $ZnCl_2$. In the same year Burgess [35] pointed out that the use of chrome in the dye bath imparts a considerable mold resistance to wool. With 0.5 percent of chrome, either as CrO_3 or Cr_2O_3, he obtained good mold resistance, and with 1.0 percent of chrome the results were even more satisfactory. In the same year Thaysen and Bunker [162] suggested the use of mixtures of iron and chromium salts for both woolen and cellulosic fabrics and recommended copper oleate. Fargher, et al. [56] summed up their long search for an ideal antiseptic for the cotton industry by reiterating that Shirlan was the best. Other excellent materials were stated to be ortho chloromercuriphenol, para acetoxymercuriacetanilide, thallium carbonate, para nitrophenol, and trichlorophenol.

Nakazawa and Takeda [117] in 1934 reported

on their efforts to control mold growth on military instruments. They found that wax containing 1.0 percent of paranitrophenol was effective for long periods. Other effective compounds were chloropicrin and para dichlorobenzene.

In 1934 McCallan and Wilcoxon [112] studied the toxicity of various compounds of many elements on various organisms. Toxicity was measured by the concentration of a particular compound that would permit only 50 percent of the spores to germinate. This concentration of a fungicide has come to be known as its minimum lethal dose (MLD). The data are interpreted in terms of the periodic system of the elements. They found that the toxicity increases toward the center of the periodic table and is less at either end. Toxicity within a group increases with increase of atomic weight. Compounds of positive elements show nearly the same toxicity, regardless of the compound used, but the hydrides of negative elements are all toxic, whereas highly oxidized forms show only slight toxicity. There is a tendency, with many exceptions, for an element that is toxic for one organism to be toxic for others. Compounds of Ag and Os are the most toxic. Other toxic elements are Hg, Cu, Ce, Cd, Pb, Tl, Cr, and As.

Lloyd [102] in 1934 found that paranitrophenol could be used to prevent mold growth in tanning liquors used in the leather industry. In the same year Burgess [37, 38] brought attention to the fact that the amounts of nutrient substances present on untreated materials greatly influences their susceptibility to mold growth. Depending on the presence of these substances, more or less of a particular fungicide is required. He recommends the use of Shirlan for cotton and wool. For wool he also recommends the use of chrome. In regard to the use of chrome, Armand [2, 3] believes that chroming removes protein degradation products from wool and thus removes the source of mold nutrition. He does not believe that chrome is fungicidal but that the wool is now "clean." He found that none of the dyes commonly used conferred antiseptic qualities on wool.

In 1937 Jarrell, Stuart, and Holman [87] found that mineral khaki dye could be made mildew resistant by the addition of a relatively insoluble copper compound. The precipitation of insoluble copper compounds on cotton duck by means of Na_2CO_3, the inclusion of copper compounds with other metal compounds in mineral-dyed cotton duck and the addition of basic $CuCO_3$ to a waterproofing solution are effective means for mildewproofing cotton duck, provided the copper content is equal to at least 0.11 percent of metallic copper, based on the weight of the finished fabric. However, for the most rigorous use a copper compound containing at least 1.7 percent of metallic copper, based on the weight of the finished fabric should be precipitated on the fabric. When chromium and iron are also used in the treating compound, somewhat less copper is required.

Neill and Travers [118] in 1938 found that waterproofing treatments on tent fabrics accelerated their deterioration by microorganisms. They recommended the use of the iron-chromium process but thought it was too expensive for general use. For practical purposes they recommend the use of a 1-percent solution of Shirlan. Schults [150] suggested that *Aspergillus niger* could be inhibited by drying materials to 20-percent moisture content and storing them at 71- to 73-percent relative humidity. If fabrics are to be kept wet for any length of time, they are best preserved with formaldehyde, Na_2SO_3, or salicylic acid.

By 1938 the use of copper naphthenate had become so widespread, especially for treating wood, that Smith [154] responded to the need for information regarding its origin. Copper naphthenate, he stated, is a combination of copper with naphthenic acids found in petroleum oil. It is soluble in kerosine, toluene, turpentine, and similar organic solvents. It is effective in the control of wood fungi, termites, fungi that attack jute and other cellulosic fibers, and even as an antifouling agent for ship bottoms. In 1939 Bryson [30] found the naphthenic acid derivatives, particularly copper naphthenate, to be most effective for preserving jute fabrics.

Everitt and Sullivan [54] in 1940 tested a large number of compounds, using five test organisms. Fungistatic activity was shown by phenyl thioarsenite, 4-chloro-2-nitrobenzene sulfonamide, sodium 1,2,naphthoquinone-4-sulfonate, and sulfanilamide. The best of the fungicidal compounds were phenyl benzothiazole and mercaptobenzothiazole. In the same year Colin-Russ [43], working with leather, found that the most efficient antiseptics, considering cost, were beta naphthol (0.1 to 0.4%), $HgCl_2$ (0.1%), and especially phenyl mercuric nitrate (0.0075%). Among those that were effective but uneconomical were paranitrophenol, glycocarvolene, para chloro-meta-xylenol, and salicylanilide. To this list Morrow and Richardson [114] added sodium pentachlorophenate. In the same year Richardson [132] tested chrome-tanned calf leather treated with various phenolic compounds. It was found that lower concentrations were needed than for vegetable-tanned leather. The best compounds were sodium trichlorophenate (0.02%), para nitrophenol (0.1%) sodium tetrachlorophenate (0.1%), sodium 2-bromo-4-6-dichlorophenate (0.1%), and pentachlorophenate (0.1%).

Furry, et al. [61] in 1941 made a study of 135 chemical treatments for the prevention of mildew in cotton fabrics. They found that fairly good protection is obtained by acetylating cotton fabric for 21 hours at 20° to 25° C., but the treatment tends to weaken the fabric. In regard to this

point, Thaysen, et al. [163], by varying the conditions and concentrations of his acetylating mixture, has been able to treat cotton without loss of strength. Among other excellent treatments they list salicylanilide in wax or resin, orthophenylphenol, 2-chloro-orthophenylphenol, and pentachlorophenol. The sodium salts of these compounds, however, are too soluble for use on fabrics.

Armstrong [6, 7] in 1941 was concerned with the mildew-proofing of jute sandbags for military purposes. For this purpose he recommends the use of (1) tar distillate or creosote (25% in fiber); (2) copper compounds, such as the napthenate, oleate, or stearate (0.8 to 1.0% of copper to be in the fiber); (3) cuprammonium solution (1.0 to 1.5% of copper to be in the fiber), (4) copper salts, such as copper chromate, aqueous colloidal copper, and copper carbonate; or (5) cutch-cuprammonium solutions in a two-bath process (0.8 to 1.0% of copper in the fiber). A minimum of 0.35 percent of copper must be present to prevent microbiological action.

In 1942 Marsh and Duske [110] found that fabrics treated with a 1 to 2,000 aqueous dilution of phenyl mercuric acetate showed effective mildew resistance even after several washings. The treatment has no apparent effect on the feel or nature of the fabric. The cost of processing is small, and tests indicate that it is nontoxic at this concentration.

Although the use of copper compounds had gained steadily, it was recognized that some were better than others. Jamison [86] in 1942 showed that copper was easily adsorbed by many soils. Consequently, if the copper ion was the only fungicidal element of a particular compound, its adsorption would leave a treated fabric or other material unprotected. On the other hand, if the copper was coupled to a radical, such as the naphthenate, which has been demonstrated to possess fungicidal capacity [108], the loss of copper by adsorption would still leave the treated material protected to a degree. However, Osnitskaya [126], in the same year, differs with this concept. She finds that naphthenic acids may serve as a source of carbon for some species of microorganisms.

Dimond and Horsfall [49] in 1943 found that, although mercaptobenzothiazole was an excellent fungicide, it was inactivated by zinc oxide. However, where it was not possible to eliminate zinc oxide from a material, tetra methyl thiuram disulfide was an excellent substitute for mercaptobenzothiazole, being entirely compatible with zinc oxide. This is especially important in the rubber industry, where zinc oxide is essential to the compounding of rubber. In the same year, Ter Horst and Felix [157] found that 2,3-dichloro-1,4-naphthoquinone was effective in the control of 22 important fungi with no harmful effects to cotton fabrics. At the same time Ordal and Deromedi [124] were reporting that two synthetic wetting agents, lauryl sulfonate and the dioctyl ester of sodium sulfosuccinate, enhanced the germicidal action of buffered solutions containing 2,4-dichlorophenol or 2,4,6-trichlorophenol. They attributed the enhancement of germicidal action to a synergistic action between the wetting agents and the undissociated phenols. Finally, in the same year Robertson [135] showed that slight concentrations of organic mercury compounds in leather had a stimulant effect on mold growth. He produced this effect with concentrations as low as 1 to 20,000 and 1 to 40,000

The year 1944 showed a sharp increase in the number of publications in the field of materiel preservation. The war in the South Pacific area had intensified research in this field. Old compounds were reexamined in the hope that improved methods for using them could be developed. Greeves-Carpenter [76] recommended the organic mercury compounds, for which Shiraeff [148] had proposed new methods of chemical determination. Lollar [103, 104] [105, 106], in a series of papers, reexamined all the available fungicides for use on leather. He tested 40 compounds and found the best were paranitrophenol, para chloro-meta-xylenol, para chloro-meta-cresol, salicylanilide, pentachlorophenol, mercaptobenzothiazole, dihydroxy dichlorodiphenylmethane, trichlorophenol, and tetrachlorophenol. He was one of the first to recommend the use of mixtures of fungicides. He found that copper and zinc compounds gave little or no protection on leather. However, copper compounds were receiving the attention of many workers in this field. Azevado and Maffei [11] reported that the most effective treatment for cotton canvas consisted in immersing the canvas in a 10 percent solution of copper sulfate, followed by immersion in a 10 percent sodium hydroxide solution. Woodford [175] warned that military textiles of cellulosic origin in the India climate required copper in some form. To increase the effectiveness of the copper compounds, he proposed the use of such binders as plasticized pitch, gear oil, and bituminous emulsion. Carter [39] was of the opinion that metallic naphthenates give greater protection to cellulosic materials against microorganisms than other metallic organic or inorganic fungicides, with which Jones [88] agreed. Jones also recommended the use of cutch followed by potassium dichromate. Finally, Marsh, et al. [108] made a study of various organic copper compounds, including copper naphthenate, copper oleate, copper tallate, and copper resinate. They showed that copper naphthenate prevents rotting of cotton fabrics by mildew at a lower concentration than any of the other compounds. They held that this was due, in part, to the fact that naphthenic acid itself is a potent fungicide. Factors that influence the fungicidal properties of the four compounds were

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claimed to be leaching, adsorption, and chemical deactivation by means of hydrolysis, and the subsequent formation of insoluble copper compounds in the soil.

In 1945 the only important new fungicides to be examined were the highly effective pyridyl mercuric compounds. Appling and McCoy [1] found that pyridyl mercuric acetate and pyridyl mercuric chloride were as effective as phenyl mercuric compounds. However, their toxicity toward *Aspergillus niger* was not as great. By this time the excellence of dihydroxydichlorodiphenylmethane (G4) had become so well established that Shiraeff [149] worked out a chemical test for its estimation. Borghetty [25] found dihydroxydichlorodiphenylmethane, phenyl mercuric acetate, and phenyl mercuric nitrilotriethanolamine to be effective fungicides. Neish and Ledingham [119] developed a new fungicide, copper dimethylglyoxime which they claimed was more effective than copper naphthenate. The compound was made by reacting dimethylglyoxime with copper acetate. Furry [63] made a study of the fungicidal properties of natural dyes and found that osage orange, quercitron, divi divi, fustic, logwood, and quebracho were all effective. Fargher [57] sums up the work of many years by saying that salicylanilide in 0.01 to 0.2 percent solution has inhibiting power equal to that of an 0.8-percent solution of $ZnCl_2$, and paranitrophenol is almost as effective. To produce great resistance in materials highly sensitive to mildew, two or more antiseptics, generally contrasted in chemical constitution and partition coefficient between water and oil should be used together, as for instance salicylanilide in combination with paranitrophenol or mercaptobenzothiazole. The rotproofing treatment should provide protection against deterioration by chemical tendering by air, light, and moisture as well as by molds and bacteria. The acetylation of cellulosic materials and the reduction of water adsorption to a very low figure by coating with plastics are applicable only to a limited extent. The success of the latter method depends upon obtaining plasticizers that resist the growth of microorganisms and deterioration by exposure to the weather as strongly as the plastics with which they are applied. The organic antiseptics used in mildewproofing are not suitable for rotproofing, since only fungicidal substances that are substantially insoluble in water are effective. Bayley and Weatherburn [19] found that the ability of cotton fabrics containing copper naphthenate to resist microbiological attack in the soil was impaired when the fabrics were leached in water for 24 hours. Chemical analysis showed that this effect was not caused by a loss of copper during the leaching but rather by a change in the copper compound resulting in a greater proportion of the organic solvent—insoluble form. The effect did not appear to be dependent on the pH of the leaching bath. In this respect it is interesting to note that in 1939, Rabanus [131], while studying the cause of the ineffectiveness of copper carbonate as a fungicide for telegraph poles, found that oxalic acid, formed by wood fungi, converts the copper carbonate into insoluble CuC_2O_4, which is ineffective as a fungicide.

As their value came to be appreciated, copper compounds received increased attention. However, it was rumored that these compounds, including the highly regarded copper naphthenate, contribute to the deterioration of fabrics on which they are deposited. Bartlett and Goll [15] found that copper sulfate under certain conditions has a tendency to promote the oxidation of cellulose. Copper naphthenate, on the other hand, does not have any deteriorating effect on cellulosic materials. They had exposed their treated fabrics to artificial ultraviolet light. Bayley and Weatherburn [20, 21], however, exposed their treated fabrics to weather conditions for a period of 7 months. They, too, found that there is no increase in the rate of deterioration because of the presence of copper naphthenate or copper oleate.

In the attempt to improve the tenacity of copper naphthenate Bayley and Weatherburn [21] in 1946 suggested the use of waterproofing materials over the treated fabric. Curwen [45] suggested that the slow-leaching out of copper naphthenate on exposed parts of treated materials could be overcome by the part-formation of aluminum naphthenate during the process of manufacture. This mixture permits the use of about 80 percent less naphthenate than is normally recommended.

VI. Testing for Fungicidal Efficiency

Strictly speaking, the ideal method of testing the efficiency of a fungus-proofing compound is exposure to severe field conditions. However, this is time-consuming and in most cases impractical for other reasons. Therefore, accelerated methods of test have been sought. The first workers in this field used empirical methods with uncontrolled conditions. Levine and Veitch [99], [168] in 1920 reviewed the subject of mildew resistance of textiles and proposed a method of testing the relative effectiveness of various treatments. They leached treated samples in running tap water for 2 or 3 days in order to wash out easily removable fungicides and fermentable matter. Then the damp samples were placed in Petri dishes containing nutrient agar jelly, and were incubated for 7 to 10 days in a dark chamber at 20° to 25° C. After this preinoculation period the samples were

inoculated with pure cultures of several species of molds and reincubated for 3 weeks to a month. They were examined each week for mold growth, and the observed conditions were rated on a scale of 10. The test was considered to be a severe one. The only treatment found to survive the full period of the test was that with copper ammonium hydroxide, the "cuprammonium process." Correlating their test with service conditions, they observed that treated canvas which gave a rating of at least six did not mildew on exposure to Washington, D. C., weather during the summer and fall months. In the same year Fleming and Thaysen [58] developed a test of mildew damage in which they treated the cellulosic material with NaOH and CS_2. Under the microscope they were able to observe and count the damaged fibers.

In 1926 Bright [28] attempted to study the extent of mildew damage by examining the fibers under the microscope. He used the Congo red test and the swelling test of Fleming and Thaysen [58]. In the following year Morris [113] tested "fabric antiseptics" for toxicity to at least eight species of molds. The efficiency of each antiseptic was compared to phenol as a standard. He found that although one mold may resist 10 times as much of one antiseptic as another species, the situation may be entirely reversed when another antiseptic is tested. Therefore, he concluded, a considerable number of species must be used in testing.

In 1929 Searle [143] described a new test method. To compare the resistance of fabrics to molds and bacteria, strips were wound around filter candles that had been covered with a thin layer of soil, and were incubated for as long as 6 weeks in a moist chamber at 30°C. They were tested for strength at periodic intervals. Searle was the first to observe the interesting phenomenon that cotton fabric deteriorated by molds has the same cuprammonium viscosity as normal cellulose.

Thaysen and Bunker [162] in 1930 relied on outdoor exposures followed by microscopic examinations. They exposed fabrics in various parts of the world, under a wide variety of conditions. In the following year Ellis [52, 53] and also Hall [79] proposed a new microscopic test method which involved the use of lactophenol and Cotton Blue. By this method mold hyphae can be differentiated from cotton fibers.

In 1934 Burgess [38] proposed a laboratory method for determining the resistance of wool and other animal fibers to bacterial action. He found that wool fibers could be disintegrated into their component elements of epithelial scales and cortical cells by exposure to an appropriately buffered solution of trypsin or pepsin. He showed that the action of the trypsin is similar to that of proteolytic bacteria but is more rapid.

In 1934 Thom, et al. [166] examined all of the available test methods and found that they fell into two classes: (1) the simulation of the field hazard under conditions more or less closely observed and recorded, and (2) accelerated methods in which the natural agents were given what were assumed to be special, favorable conditions to attack the fabric and thus to concentrate into a short period the destructive effects normally extending over long periods. In both procedures, after incubation, it was necessary that some kind of laboratory examination be applied to give comparative values to the measurement or estimation of the stage of deterioration. They considered only the effect of cellulolytic organisms. After investigating the possibilities of a test based on a mixed culture inoculum they came to the conclusion that only a pure culture inoculum could be expected to give reproducible results. This decision necessitated the selection of a suitable test organism and an appropriate medium. They set up the following requirements for a suitable test organism:

1. Capable of rapid destruction of cotton fabrics.
2. Must be easy to cultivate.
3. Must be adaptable to a fairly wide range of conditions.
4. Must be easy to identify in order to maintain in pure culture or to free from contaminants.

Thom. et al. selected a modification of Czapek's agar medium similar to that used by Dubos [51] in 1928 but differing in that 3.0 g of $NaNO_3$ per liter was used in place of 0.5 g per liter and a pH of 6.8 in place of 7.5. *Chaetomium globosum* [40] was found to be the most destructive of the molds tested. It had been reported previously as a cellulose destroyer by Galloway [64] in 1930, by Thaysen and Bunker [161] in 1927, and by others. When tested against other species of *Chaetomium*, it was found to have the greatest cellulolytic power. This was confirmed by Greathouse and Ames [75] in 1945. In figure 5 can be seen *C. globosum* growing on lacquer-impregnated filter paper.

In 1940 Rogers, et al. [136] made an intensive study of the effects of fungal growth on cotton fabrics. As test organisms they selected *Chaetomium globosum* and *Spirochaeta cytophaga*. They reaffirmed the excellence of the choice of *Chaetomium globosum* as a test organism and observed that *C. globosum* is unique in that the pH of the medium is 6.8, both before and after completion of the incubation period.

Everitt and Sullivan [54] in 1940 tested fungicides by adding them directly to the growth medium. They distinguished between fungistatic and fungicidal compounds. In the same year O'Flaherty and Doherty [123] reported that there was no standard accelerated test for evaluating fungicides for leather. Several pages farther in the same journal, Richardson [132] reported that he had sprayed treated squares of leather with a

FIGURE 5. *Growth of Chaetomium globosum on filter paper impregnated with a lacquer containing a fungicide.*
The growth of *Chaetomium globosum* on the specimen indicates an inferior treatment. (Note bacterial contamination of agar.) (Unsterilized specimen used.)

suspension of mixed mold spores. These were stored damp at room temperature for 50 days. The time required for visible mold growth on untreated squares was 6 to 9 days.

In 1941 Downing and Whitmore [50], speaking for the leather industry, observed that laboratory tests are not altogether reliable because molds gradually acquire immunity to fungicides. It should be pointed out that this objection does not apply to pure culture tests in which the organisms come in contact with fungicides only once and are then discarded. Downing and Whitmore are referring to mold chambers that are used constantly for testing fungicides. To prevent the molds from acquiring immunity, they recommend that different fungicides be tested alternately.

Greathouse, et al. [74] in 1942 made some noteworthy contributions to the pure-culture test method. Rogers, et al. [136] had recommended the use of square 16-ounce screw-cap bottles for incubation chambers. Greathouse, et al., greatly improved them by modifying the metal cap. A hole was cut in the cap and was covered with a piece of closely woven glass fabric. This served two purposes: (1) It became easier to maintain sterility, and (2) better aeration of the incubating specimen was possible. Another modification was the substitution of a glass wick for the agar that had formerly been used. They also held that the adjustment of media to a given pH with HCl or NaOH before autoclaving was valueless, as after autoclaving the pH of adjusted and non-adjusted media becomes approximately equal. From results obtained with a variety of dilutions of inoculum, they concluded that the concentration of inoculum, within rather wide limits, does not seem to affect the degree of growth after 14 days of incubation, and for this reason it is not necessary to specify the concentration of a spore suspension with any great exactitude. In addition to *Chaetomium globosum*, they worked with a new organism, *Metarrhizium sp.*, which was later named *Metarrhizium glutinosum* by Pope [128] and which has been identified as *Myrothecium verrucaria* by White and Downing [172]. They considered *Myrothecium verrucaria* to be superior to *Chaetomium* as a test organism. Their outstanding contribution, it seems, was their insistence on buffered media.

By 1943, soil-burial tests had achieved a degree of popularity. Many workers were realizing the limitations of the pure-culture test methods and

were seeking a more severe test method. Furry and Zametkin [62] developed the soil-suspension test. Test specimens were inoculated by immersion in a soil suspension made from composted soil. The inoculated specimens were incubated on agar medium, glass wick-nutrient medium, or even cotton-batting in nutrient medium, all in 16-ounce square bottles. They claimed excellent differentiation of fungicides, approaching the soil-burial test in potency. Borlaug [26] recommended the use of the soil-burial test for synthetic fabrics in preference to pure-culture methods. And Bunker [31] suggested the use of aluminum frames for mounting soil-burial test specimens. These were buried vertically in typical light garden soil and kept at 30° C and 20-percent water content. They were tested weekly by attempting to tear them by hand. Finally, Bertolet [23] decided that as a result of extensive work in testing the mildew resistance of jute and osnaburg fabrics used in sandbag construction, the laboratory-accelerated mildew test should be abandoned in favor of the soil-burial test as a measurement of the service life of this material.

In 1944 Lollar [103, 104] proposed the use of mixed cultures of molds for testing mildew-proofed leather. ZoBell and Beckwith [178] made a similar recommendation for testing rubber compounds. Fabric testing was also showing a return to less controlled test procedures. Azevado [11] described an accelerated rotting test in which test strips were interlayered with strips of rotten canvas. The incubation period was 60 days under tropical conditions. However, such workers as Marsh, Greathouse, and Barker were still carrying on the fight for standardized, controlled test methods. Marsh, et al. [108] in testing a variety of copper compounds, concluded that laboratory test methods had not yet developed to such a stage that their results alone could be used as accurate predictions of the resistance to mildewing or rotting of a treated fabric under all of the varied service conditions to which it might be subjected. Therefore, they tested treated fabrics by simulating field conditions before subjecting them to pure culture tests. These modifying tests included: leaching, adsorption by bentonite, and chemical deactivation by means of hydrolysis. They also made use of a copper-tolerant mold, *Aspergillus niger*. They emphasized the futility of attempting evaluation by the use of mixed cultures.

Klemme, et al. [96] in 1945 examined 43 organisms for cellulolytic activity. Of these 29 caused more than 50 percent loss in breaking strength of cotton duck when incubated for 7 days on a liquid mineral salt medium that contained NH_4NO_3 as the nitrogen source. Also, they studied the effect of using different sources of nitrogen. Whereas most of the organisms grew best when NH_4NO_3 was the nitrogen source, *Chaetomium globosum* was unique in that it grew best on $NaNO_3$. This was later verified by Greathouse and Ames [75], who studied the cellulolytic acticity of 16 species of *Chaetomium*. They found that *C. globosum* was the most active member of this genus. The ability of this group to decompose cellulose was found to vary with the source of nitrogen, the pH, the temperature, and with the fabric preservative.

Up to 1945 the requirements for leaching specimens before a mildew test were more or less haphazard. Thom, et al. [166] had suggested that fabrics be soaked in several changes of water or in running water for 2 days. Several Government procurement specifications called for a leaching period of 30 hours in running tap water. Furry and Zametkin [62] suggested a 24 hour leaching period and specified that for each ounce of fabric there should be 1 gallon of water with three complete changes during the 24 hours. Goodavage [71] described two types of tests: a spray test for water repellent materials, and a leaching test. For the leaching test he specified that there be 1 gallon of water at 65° F for each specimen 6 inches by 12 inches and suggested that there be three changes of water in 24 hours. In 1945, Marsh, et al. [109] developed an apparatus that could deliver water at a temperature of 30° C to quart Mason jars. The flow was so regulated that there were 10 changes of water per hour. Also, they considered the effect of steam sterilization on organic fungicides and concluded that it is, perhaps, an unnecessarily severe test for these fungicides. When a nonsterile technique is required, as in the case of the steam-distillable organic fungicides, they found that *C. globosum* was more satisfactory than certain other forms as a test organism as it is less apt to be contaminated. They recommended a filter paper-mat technique in which the test fabric was planted on a mat of mycelium. The mycelium was induced to grow on a filter-paper strip supported on a mineral salts-agar medium. This type of test was found to be much more severe than the previously described pipette inoculum procedure. They believed that the choice of the best test or combination of tests to determine mildew resistance of a fabric depends on the service conditions under which the fabric is to be used.

In the same year Dean, et al. [46] reported on extensive studies of the soil-burial test method. They used only composted soil and carefully controlled the temperature and moisture. To obtain reproducible results they found that it was necessary to use fresh soil for each test. The soil-burial resistance for cotton osnaburg treated with copper naphthenate was measured for 10 identical tests and was found to be reproducible with a coefficient of variation of 8.5 percent.

In 1946 Romano [137] reexamined the role of

nitrogen in the growth of *Chaetomium globosum*. Waksman and Hutchings [169] had shown that a ratio exists between the activity of cellulose decomposing microorganisms and the amount of available nitrogen present. The higher the cellulose content of soils, the greater is the need for nitrogen to maintain cellulolytic activity. Romano used NH_4NO_3 because it maintained a more stabilized pH than $NaNO_3$ or KNO_3. He found that the optimum concentration of nitrogen was 1.4 to 1.75 grams per liter of medium. When the concentration of nitrogen was increased as high as 7.35 grams per liter, there were no toxic or inhibitory reactions. Varying the other elements in the medium had no effect on growth. He concluded that the nitrogen concentration of the nutrient media in general use was insufficient for maximum growth.

Part 2. Investigation of Microbiological Tests

VII. Development of Mildew Testing at Bureau

The primary problem of testing for mildew resistance at the National Bureau of Standards has been the development of tests that are reproducible and sufficiently severe to give that margin of safety that is required for tropical use.

Considering the great number and variety of fungicides, it is obvious that they cannot be equally effective, especially as it has been shown that they act in a variety of ways. In order to determine their relative effectiveness, it is necessary to have methods of bioassay. The term "bioassay" is stressed because chemical analyses alone are not sufficient. It can easily be ascertained that a particular fungicide is effective in a particular concentration. Why, then, is it not sufficient to make a quantitative analysis of a treated material to learn whether the desired concentration has been deposited? First, chemical analysis cannot determine the degree of penetration of a fungicide or its distribution over the treated material. Second, as has been pointed out previously, different materials react in decidedly different ways to fungicides. For example, fungicides that are effective for cotton may not be effective for leather. Furthermore, it is important to test a material with regard to its ultimate use. A material that is to be protected only during storage need not have as permanent a fungicide as a material that is to be exposed to the elements and which may come in contact with the ground.

Many workers have attempted to develop test methods for the evaluation of fungicide-treated materials. The first to tackle this problem were those interested in the preservation of cotton textiles. Fabrics to be tested were inoculated from rotted fabrics or from composted soils. No effort was made to determine the nature of the organisms used or to accelerate the test by adding nutrients. Within the past decade Thom and his coworkers have reexamined the question of mildew tests and have decided that any test that makes use of mixed cultures of organisms does not lend itself to scientific accuracy. Thereupon, they studied the growth habits of many cellulose-destroying organisms. They chose an exceptionally stable organism *Chaetomium globosum Kunze* which, during 5 years of continuous subculturing in the Microbiological Laboratory at the National Bureau of Standards, has not been observed to change its growth characteristics. It grows well on a mineral salts-agar medium when a cellulosic carbon source is provided. When growing the organism for stock cultures, filter paper is the usual source of carbon. When testing a cotton fabric, the test material itself supplies the carbon. If the specimen does not contain a fungicide, *C. globosum* will utilize the cellulose as a carbon source. If, however, the fabric is adequately mildew-proofed, the organism will either be poisoned or the cellulose will not be available as a source of carbon, depending on the mildew-proofing agent used.

As originally proposed by Thom and his coworkers, the culture medium was a modification of that proposed by Czapek:

$NaNO_3$	3.0 g.
K_2HPO_4	1.0 g.
$MgSO_4 \cdot 7H_2O$	0.25 g.
KCl	0.25 g.
$FeSO_4$	0.01 g.
Agar	10.0 g.
Distilled water	1.0 liter.

Adjust pH to 6.8 with NaOH or HCl as required.

This medium was originally developed for mycologists in order to study the growth characteristics of various organisms. For this purpose a rather sparse growth is preferred. Therefore, by using Thom's medium, which does not give maximum growth, *C. globosum* was not being used to fullest advantage as a test organism. In brief, the test method was as follows: strips of treated fabric of a size suitable for determining breaking strength were sterilized and laid on a surface of mineral salts-agar in bottles under aseptic conditions, after which they were inoculated with an aqueous suspension of the spores of *C. globosum*. Untreated fabric was given the same test to serve as a control. Next the bottles were incubated for 14 days at a temperature of 28° C, after which the specimens were removed and washed, and, when dry, their breaking strength was determined. Of course, the untreated fabric was completely

degraded. If a fabric retained 90 percent of its original strength, it was considered to be adequately mildew-proofed.

Shortly before the outbreak of the recent war it became necessary to test the mildew-resistance of sandbag fabrics. As a better method was not available, Thom's test was used. However, its shortcomings soon became apparent. The optimum pH of 6.8 could not be maintained and it was found that certain fungicides inhibit *C. globosum* at concentrations far less than that necessary for effective mildew-proofing. For example, it is known that copper naphthenate, for effective mildew-proofing, must be in fabric to the extent of 0.8 percent of metallic copper, based on the finished weight of the fabric, yet 0.3 percent will inhibit *C. globosum* in this test.

By observing the visual appearance of growth from day to day, it was noted that the degree of growth, as seen with the unaided eye, was a good measure of the physical break-down of the fabric. If there was no growth by the seventh day, there was none by the fourteenth day. Therefore, after 35,000 strips had been observed, the test was shortened to 7 days, and breaking strength determinations were discontinued. However, this did not increase the severity of the test. It could not be used for the evaluation of new fungicides. It was used mainly in procurement testing of approved fungicides for army matériel. A chemical analysis of the treated fabric was first made and if there was sufficient fungicide present, a *Chaetomium* test was given to determine the uniformity of treatment application.

While this test was being used a concerted effort was being made to improve the cellulolytic activity of *Chaetomium*. By experimenting with a variety of media, some of which had been suggested by Greathouse and Marsh, a medium, double-buffered with K_2HPO_4 and KH_2PO_4 was selected (see page 25).

According to Romano [137] this medium, containing 3.0 grams of NH_4NO_3, has the optimum nitrogen content (1.6 g/liter) for *C. globosum*. Furthermore, the pH is close to 6.8 and requires no adjustment. Although Greathouse and Ames [75] have shown that $NaNO_3$ gives slightly more breakdown of cellulose with *C. globosum*, it has been considered advisable to use NH_4NO_3 because of greater stability of pH due to buffering action and also because this medium can be used for *Aspergillus niger* by adding 30 grams of sucrose per liter as a carbon source. Inasmuch as tap water has been specified, no trace elements need be added. However, in localities where the tap water is not close to neutrality, or where it contains large quantities of minerals, distilled water should be used with the addition of the necessary trace elements.

Although this medium improved the reproducibility of the test, it was still not the ideal test. On the basis of work by Greathouse and Marsh, the test known as the *Chaetomium* mat method was standardized. It is more potent than the *Chaetomium* method of Thom but is not quite as severe as the soil burial test. The test makes use of the double-buffered salts-agar medium (page 25). A strip of sterile filter paper is placed over the medium and is inoculated with *C. globosum* spores under aseptic conditions. After 3 days of incubation, a dense white mycelium develops over the surface of the filter paper. This is the mat that gives the test its name. At this point an unsterilized test specimen is dipped in a fresh inoculum and placed over the mat, which is incubated again for 14 days. Even copper naphthenate-treated fabric loses some of its strength in this test. The loss is roughly comparable to that of a 6-week soil-burial period.

Up to this point the emphasis has been on cellulosic materials. Historically, cellulosic materials were the first to be considered from the point of view of microbiological deterioration. Early in the recent war, however, many reports were received from tropical battlefields that not only cellulosic materials but a wide variety of materials was being attacked by fungi—woolen blankets, leather shoes, and instrument cases, all types of plastic materials, rubber (synthetic and natural), and even the lenses of binoculars and cameras. As cellulose was rarely a component of any of these products, *C. globosum* was obviously unsuited as a test organism. Furthermore, the organisms that attacked these materials in the field were usually surface growers that caused damage in other ways than loss of strength. Consequently, an entirely new philosophy of mildew testing was developed. The measure of mildew resistance in this type of testing is the degree of growth of the test organism rather than the determination of its effect on some desirable property of the test specimen as, for example, breaking strength in the case of cellulosic materials.

Mildew-resistant camouflage paints were the first noncellulosic materials to be tested in this laboratory. Inasmuch as *Aspergillus niger* is such an ubiquitous and prolific organism, several strains were obtained from the United States Department of Agriculture, and the one that is now a standard test organism was finally selected. The medium chosen was the double-buffered modification of Czapek's containing 30 grams of sucrose per liter. The organism was able to grow on this without obtaining nourishment from the test specimen. However, if the specimen did not contain a fungicide, the organism would invariably overgrow it. Figures 6 and 7 illustrate the *A. niger* test.

Aspergillus niger has been found to be a very useful test organism. Although it was originally selected for testing the mildew resistance of camouflage paints, it was found that it could be

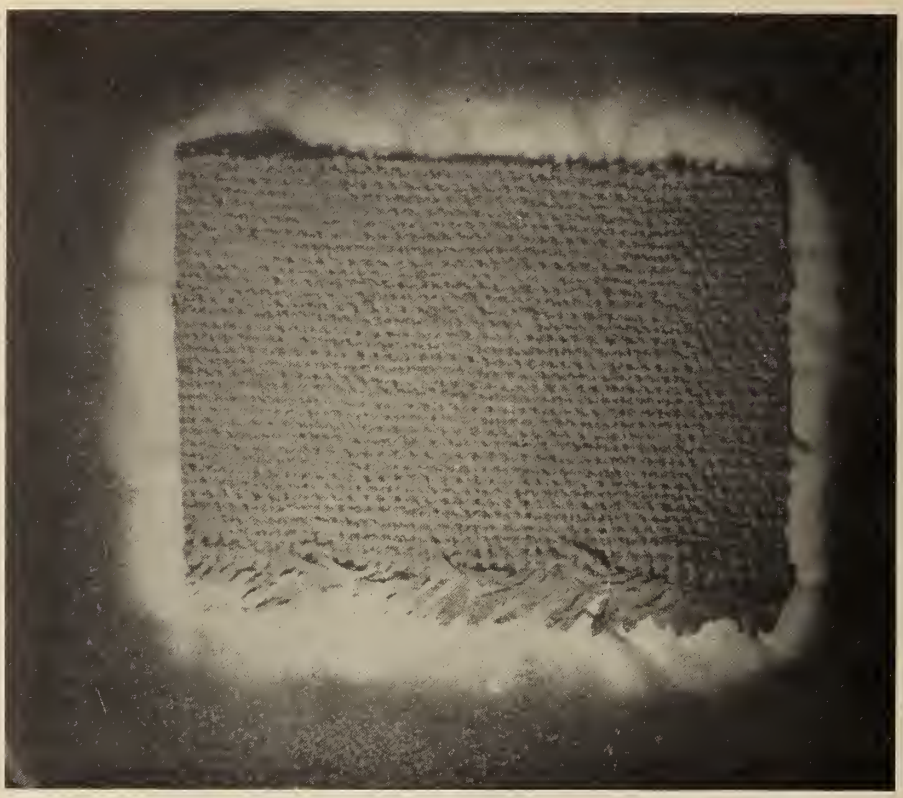

FIGURE 6. *Mildew-resistant camouflage fabric on Aspergillus niger plate.*
Specimen completely inhibits growth of organisms.

used as a test organism for all materials which, in the field, supported a growth of the prolific surface growers, as for instance leather. Vegetable-tanned leather contains all the necessary nutrients for the growth of *A. niger*. Although it is not necessary to use a mineral-salts-sucrose agar medium in testing leather, a greatly accelerated test is obtained when it is used. Other materials that have been tested for mildew resistance by the use of *A. niger* are woolen fabrics, rubber (natural and synthetic), plastics, and plasticizers.

The medium used for all tests in which *A. niger* is the test organism is the same as the double-buffered *C. globosum* medium (page 25) with the addition of 30 grams of sucrose per liter. The test is as follows: A sterile culture plate is inoculated with the spores of *A. niger* and is incubated for 48 hours at 28° C and a relative humidity of 90 to 95 percent. At the end of this time the surface of the agar is completely covered with white mycelium. Thereupon the treated specimen is moistened and placed over the center of the mat and incubated again for 7 days. At the end of this time an adequately mildew-proofed specimen will be entirely free of growth and may even have a white zone of inhibition around it. Beyond the specimen, however, the white mycelium has turned to black spores. The untreated control on the other hand will be completely covered by the black spores.

The *A. niger* test has been modified slightly to test the mildew susceptibility of materials such as rubbers and plastics, which do not necessarily contain fungicidal substances. The object is to determine whether these materials can support a growth of mildew. Therefore, the sucrose is omitted from the medium. As the organism cannot grow on this incomplete medium, the test sample is first placed on the agar medium and then is inoculated by pipette, as in Thom's *Chaetomium* test.

As stated previously, fungicides cannot be tested apart from their existence on organic materials. Although many compounds are extremely toxic to microorganisms in solution, their usefulness on fabrics or other organic materials may be limited by their excessive solubility, their activity as catalysts in the photochemical deterioration of cellulose, or by their instability to ultra violet light. Therefore, any evaluation test method must include the resistance of the treated material to micro-organisms under a variety of conditions. To be more explicit, a treated fabric may be tested with a pure culture of a particular mold that is known to have cellu-

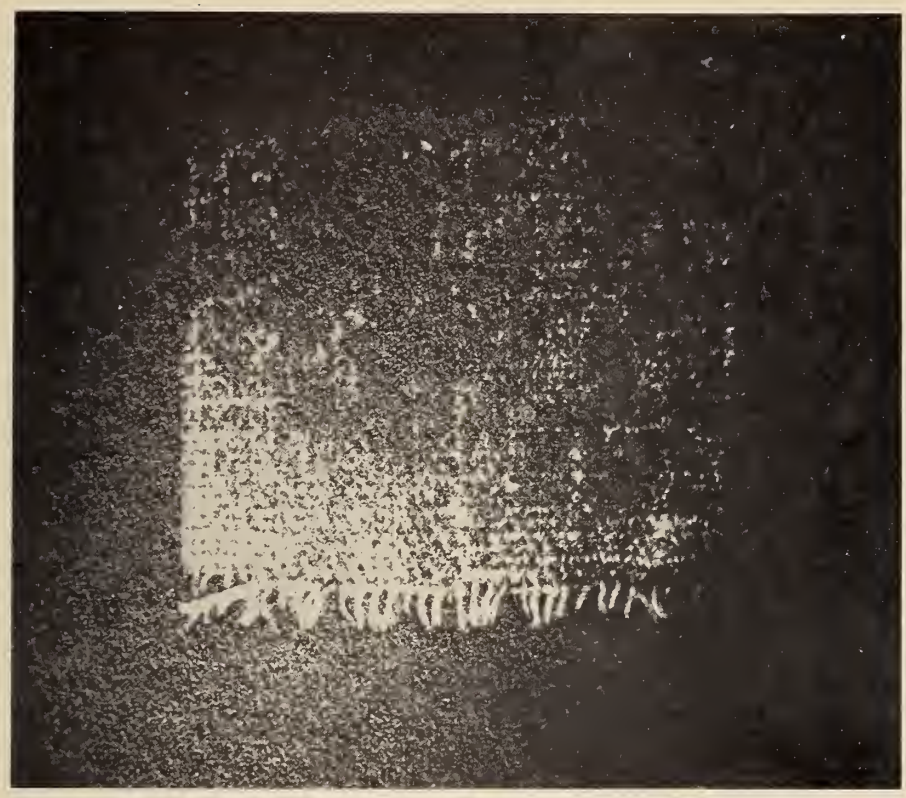

FIGURE 7. *Camouflage fabric without fungicide on Aspergillus niger plate.*
Specimen heavily invaded by organisms.

lolytic power. In a test of this nature the treated fabric may show no growth of mildew and may retain all of its strength, whereas an untreated control specimen may be completely degraded. This merely indicates that the particular fungicide is of sufficient concentration to prevent the test organism from developing. It tells nothing about the permanency of this fungicide. A second test which then suggests itself is one that seeks to determine the solubility of the fungicide as it exists on the treated material. Washing the treated material in running tap water for 24 hours has proved to be adequate for most treated materials. As a rule, however, treated leathers are leached for 48 hours because the fungicides are usually combined with oils and greases, which tend to decrease their solubility. Copper sulphate, which is an admirable fungicide, is easily leached out in a test of this nature.

Fungicides that successfully pass a mildew test after 24 hours of leaching are next subjected to artificial weathering conditions in which the combination of ultraviolet light and moisture are brought to bear on the treated material for a definite period of time, such as 360 hours, depending on the type of weathering machine used. Certain chemicals, such as manganese compounds, are known to act as catalysts in the photochemical deterioration of cellulose. Others, such as the chlorinated phenols, and especially pentachlorophenol, break down in the presence of ultraviolet light and liberate HCl, which attacks fabric and weakens it considerably even before being subjected to the action of molds. In order to determine whether a fungicide has weakened a fabric during ultraviolet light exposure, breaking-strength determinations must be made after exposure and before mildew tests are attempted. A loss of more than 10 percent in strength is usually indicative of photochemical degradation. However, a fungicide may break down as a result of an artificial weathering test without damaging the fabric. An illustration would be a buffered pentachlorophenol treatment in which the HCl is neutralized as it is formed. A subsequent mildew test would then show the lack of fungicidal activity.

Although a series of tests such as has been outlined would seem to be sufficient to rank fungicidal treatments according to their efficiency, that, unfortunately is not the case. The use of artificial weathering followed by pure-culture inoculation tends to divide fungicides into two groups: those that resist the effects of light and water and those that do not. Furthermore, it does not rank the effective treatments in order of

excellence. By this method a fungicide is either good or bad. But experience has shown that all good fungicides are not equally good, and some of the bad ones have their uses.

Inasmuch as the military matériel, for which these investigations were undertaken, were to be used under the most rigorous conditions, a very severe test was desired in order to assure a wide margin of safety. Field exposures were time-consuming and otherwise impractical. A practical solution to this problem has been the adoption of the soil-burial test. This is the most potent microbiological test method in use at the present time. Top soil or compost is kept in small containers in a cabinet in which the temperature and humidity are carefully controlled. Originally, the results obtained with this test were unusually variable, even for a biological test. However, it has been found that if the temperature and mositure are carefully maintained, the results are much more reproducible. As in all other biological tests, untreated material of the same type as the treated test sample is tested at the same time to show that organisms responsible for deterioration are active. Inasmuch as this test was so variable, another control was considered necessary. It was decided to choose one fungicide that was invariably effective and to use it as a reference standard for each new fungicide tested in the soil beds. In this way if there were any variations in results due to variations in the conditions of the soil bed, they would be reflected in the standard samples. The unknown could then be evaluated in terms of the standard. This standard was, and still is, copper naphthenate in a concentration of 1 percent of metallic copper, based on the finished weight of the fabric. Strangely enough, many of the fungicides that broke down during artificial weathering proved to be excellent in the soil-burial test. Among the best of these were the chlorinated phenols, especially pentachlorophenol and dihydroxydichlorodiphenylmethane. Either of these compounds might be recommended for materials that were not to be used in direct sunlight, as, for example, in stored materials. On the other hand, many compounds that had successfully passed the accelerated weathering test plus a pure culture test were quickly degraded in the soil. These were chiefly mercury compounds and the more soluble chlorinated phenols. Such compounds might well be used for materials that would not normally come in contact with the ground as, for example, stored articles. Again, this is a test that divides treatments into two distinct classes. Although there is a slight tendency for ranking of the superior treatments, it is not entirely correlated with the ultimate use of the material. The main virtue of this test is the wide margin of safety it permits when one relies on its results, especially for treated materials that are to receive most severe exposure as sandbags and tents in tropical regions.

VIII. Representative Problems

The following investigations are representative of the microbiological research and development conducted at the National Bureau of Standards during the past 5 years. The problems are numbered from 1 to 10. This same numbering scheme is followed in the discussion starting on page 29 and in the summary beginning on page 38.

1. In developing the *Chaetomium* test, which had been first suggested by Thom and other authors [166] it became necessary to know the rate at which cellulosic materials deteriorate under standard conditions, and whether cellulosic materials of different origins break down in the same manner. For experiments to obtain this data, untreated cotton osnaburg (a coarse, unbleached cotton fabric), having an average breaking strength of 73 pounds for 1 inch-wide specimen, and jute burlap, having a breaking strength of 121 pounds for a 2-inch-wide specimen, were used. To reduce the inherent variability of jute burlap, which has only 12 threads per inch, 2-inch raveled specimens were used. Eighty specimens of each type of fabric were sterilized for 1 hour in the autoclave. Thereupon, 10 specimens of each sterilized fabric were set aside for breaking-strength controls. The remaining specimens were laid on double-buffered mineral salts agar and were inoculated with 2 milliliters of inoculum prepared in accordance with the method outlined under *Chaetomium* test procedures (page 25). The specimens were then incubated at 28° to 30° C and a relative humidity of 90 to 95 percent. Ten specimens of each fabric were removed after 3, 4, 5, 7, 8, 9, and 10 days. After conditioning at 70° F and 65-percent relative humidity, they were broken and were compared with the controls. In table 1 the results are listed as strength retained in percentage of the original strength.

2. Inasmuch as cotton fabrics of different thicknesses, treated with equal concentrations of the same fungicide, showed varying resistance to degradation in the soil-burial test, it was decided to investigate the effect of thickness of fabric on the rate of deterioration in the soil-burial test. For this investigation four fabrics were selected. They were in order of increasing thickness: a 4-ounce lightweight sheeting with a breaking strength of 46 pounds per inch width, a cotton osnaburg having a breaking strength of 84 pounds per inch width, and a 20-ounce duck having a breaking strength of 219 pounds per inch width.

Inasmuch as these fabrics were all fairly tightly woven, it was considered advisable to select a loosely woven fabric in order to determine the effect of tightness of weave on microbiological deterioration. For this purpose a 37.5-ounce loosely woven duck, having a breaking strength of 499 pounds per inch width, was chosen. Fifty specimens of each fabric were buried in the soil beds in a randomized pattern to eliminate the effect of possible variations in the soil bed. After 6, 10, 14, 21, and 28 days, 10 specimens of each sample were removed, conditioned and broken. The results in table 2 are listed as strength retained in percentage of the original strength.

3. The manner in which specimens are buried in the soil-burial test has varied from laboratory to laboratory. Bertolet [23] favored horizontal burial at a depth of approximately ½ inch. On the other hand, this laboratory preferred vertical burial to a depth of 4 inches. To test the efficiency of these two methods, cotton osnaburg having a breaking strength of 79 pounds per inch width was used in comparative tests. Sixty specimens were buried vertically and sixty specimens were buried horizontally. Ten specimens of each were removed after 2, 4, 5, 7, 8, and 9 days and were conditioned and broken. The results in table 3 are listed as strength retained in percentage of the original strength.

4. Although relatively small concentrations of copper compounds were known to inhibit *Chaetomium globosum* under laboratory conditions, their resistance to leaching from fabric in the field was not known. In order to recommend the concentrations of each fungicide that would not only be effective from the start but would have a safety factor for prolonged effectiveness in the field, it became necessary to determine the permanence to leaching of the superior copper fungicides. For this investigation cotton osnaburg strips were impregnated with copper naphthenate, cuprammonium, and copper ammonium fluoride, all in a concentration of 1.0 percent of metallic copper, based on the finished weight of the fabric, except that for cuprammonium the copper requirement was 1.5 percent. Lower concentrations had previously been eliminated by failure in the soil-burial test. Twenty specimens of each of the treated fabrics were extracted with distilled water in a Soxhlet apparatus at a temperature of 70° C. After 24, 48, 72, and 96 hours of extraction, specimens of each fabric were removed and given a qualitative *Chaetomium* test. The results of this experiment are listed in table 4.

5. From various sources there had been conflicting information regarding the activity of copper compounds as catalysts in the photochemical degradation of cellulose. Inasmuch as copper naphthenate was the most important of the copper fungicides, an experiment was set up to determine the effect of various concentrations of this compound on cellulose in the presence of light. Cotton osnaburg, having an original breaking strength of approximately 85 pounds, was impregnated with copper naphthenate in Stoddard's solvent in five concentrations. The concentrations (based on metallic copper, in the copper naphthenate, as percentage of the finished weight of the fabric) were 0.5, 1.0, 2.0, 3.0, and 4.0 percent. Although copper naphthenate is rarely used in fabrics in greater concentration than 1.5 percent, for the purposes of this experiment it was considered advisable to work with as wide a range of concentrations as possible.

Two types of light exposure were used. In one (see table 5, a) the samples were hung in a weathering machine equipped with Sunshine carbons. The apparatus consisted of a vertical carbon arc mounted at the center of a vertical cylinder. The panels of Corex D filter glass, which normally surround the carbon arc, were removed for this experiment to increase the intensity of the ultraviolet rays. The alternating-current arc was operated on 60 amperes and 50 volts. The samples were mounted on a rotating rack inside the cylinder. The distance from the center of the arc to each specimen was 18¾ inches. The rack rotated about the arc at a uniform speed of about 1 revolution in 2 hours. The samples received a fine spray of water once during each revolution of the rack. Specimens of each sample were removed after 50, 100, and 150 hours. The specimens were raveled to an equal thread number approximating 1 inch in width and were broken. The copper content of the broken strips was determined. The data are reported in table 5, a. Each figure for residual strength represents an average of 10 specimens. The figures for copper content are based on three determinations for each average.

In the second type of light exposure, 10 strips of each concentration and 10 strips of untreated fabric measuring 8 inches in the warp direction by the full width of the fabric, which was 36 inches, were mounted loosely on wooden frames on the roof of the National Bureau of Standards in Washington, D. C. Each month thereafter one strip of each concentration and a strip of untreated fabric were removed from the racks and given the same analyses as the samples that had been exposed to ultraviolet light. The results appear in table 5, b.

6. Practically all of the better known mildew-proofing agents have been evaluated by a large number of laboratories by the use of one or more test methods. In the effort to determine the relative effectiveness of the most popular test methods an ambitious investigation was undertaken. Ten-ounce cotton duck, having an original breaking strength of approximately 145 pounds, was impregnated with 35 of the most commonly used mildew-proofing agents. The compounds used are listed in table 6. Each compound was

applied to the fabric in three concentrations. The middle concentration of each compound is either the one that is most generally used, or where this information was lacking, it represents the concentration that is most likely to give good protection under some but not all of the test conditions.

The test methods (see page 25) selected for this investigation include

a. *Aspergillus niger*—mycelial mat test.
b. *Chaetomium*—direct inoculation; sterilized specimen, qualitative test.
c. *Chaetomium*—mycelial mat test.
d. Accelerated artificial weathering—360 hours.
e. *Chaetomium*—mycelial mat test after 360 hours accelerated artificial weathering.
f. *Pencillium* 66 (USDA)—mycelial mat test.
g. Soil-suspension test.
h. Soil-burial test—3, 6, and 9 weeks.

Marsh and Greathouse recommended the use of *Pencillium* 66 for two reasons: (1) it is an active cellulolytic organism, and (2) there were indications that it is not inhibited by mercurial fungicides. Therefore, it was decided to test it in comparison with *Chaetomium globosum*, which is inhibited by such fungicides.

In the visual tests 5 specimens were used for each operation, and 10 specimens were used for the breaking-strength determinations. The results, as they appear in table 6, are thus the average of 5 observations in the qualitative tests and 10 determinations for the quantitative tests.

7. In order to determine whether molds derive nourishment from the protein substance of vegetable-tanned leather or from the oils and greases that are added during manufacture, a large sample of vegetable-tanned leather was degreased by extraction with Stoddard's solvent. By this method none of the water-soluble materials was removed.

The degreased leather was divided into specimens measuring 2 inches by 2 inches. Five specimens were tested for resistance to *Aspergillus niger*. Of the remaining specimens five were treated with fatty oil, five with leather grease, five with fatty oil plus 1 percent salicylanilide, and five with fatty oil plus grease plus 1 percent salicylanilide. All of the modified specimens were also tested for resistance to *Aspergillus niger*. The results appear in table 7.

8. In a series of cooperative tests of leather in which three other laboratories participated, it was shown that the *Aspergillus niger* test, developed at the National Bureau of Standards, was as reliable as the mixed-culture tests developed by other laboratories. In some instances the *A. niger* test was more severe than any of the other tests. The test is outstanding for its reproducibility and for its speed, requiring only 7 days for completion. This test was used in the evaluation of a large number of leather treatments of which the most satisfactory, 11 in number, are presented in table 8.

Vegetable-tanned leather squares measuring 2 inches by 2 inches were impregnated with four concentrations of the 11 compounds dissolved in Stoddard's solvent. Although the concentrations of each compound cover a wide range, they are not evenly spaced. The great absorptive power of leather makes control of application very difficult. However, it is believed that the concentrations within each treatment are spaced far enough apart to demonstrate the level at which mildew resistance is effective.

9. The entire program of research in the field of the mold susceptibility of plastics, which has been reported by Brown [29], was initiated at the National Bureau of Standards as a result of an accidental observation. Before the need for mildew-proofing of plastic materials was recognized, it was observed that different samples of vinylite-coated cotton fabrics showed varying responses to test organisms. Some supported a vigorous growth, whereas others did not. To the eye there was no apparent difference in these materials. As they were supplied by different manufacturers, and as there was no variation in the results of any one manufacturer's product, the basic components of these vinylites were obtained from each processor. It was then found that the components from the various plants differed in only one respect, namely, the plasticizer. All used the same vinyl chloride-vinyl acetate copolymer, but there were two plasticizers in use. Some used tricresyl phosphate, which was demonstrated to be mold resistant. The coated fabrics, which had consistently supported a growth of mildew, were found to contain a derivative of ricinoleic acid that was extremely susceptible to mold growth. Thereupon, a study of 15 pure plastics (polymers) and 83 plasticizers was undertaken. The polymers were prepared in the form of 2 inch by 2-inch squares without plasticizer by the members of the Plastics Section, National Bureau of Standards. These specimens were tested both in the complete medium-*A. niger* test and the sugar-free *A. niger* test. As the plasticizers were, in all cases, liquids of varying consistency, a modified technique was developed. Glass rings, ½ inch in length by ½ inch in diameter were fitted to the center of Petri dishes. The sterile agar was poured into the Petri dishes in such a manner that the rings were surrounded, but not invaded, by agar medium. In the case of the sugar-free test, the plasticizer was poured into the cylinder before inoculation. In the complete culture medium test the agar around the cup was permitted to develop a mycelial mat, after which

the cup was filled with plasticizer. The results are reported in table 9, a and b, and are averages of five observations for each test.

10. The study of the susceptibility of plastics to mold growth stimulated the investigation of the mold susceptibility of a group of related materials. These included unvulcanized rubber (natural and synthetic), nylon fabric, glass fabric, and mineral wool. The results are reported in table 10 and are averages of five observations for each test.

IX. Test Methods

In the foregoing description of investigations, the test methods are outlined briefly. At this point it is desirable to set forth in greater detail the techniques of the methods used. The types of biological test methods fall into two general classes, namely, pure-culture and mixed-culture methods. In the pure-culture methods, *Chaetomium globosum* is used to measure the rot resistance of cellulosic fabrics and *Aspergillus niger* is used to test the mildew-resistance of all noncellulosic materials. The tests are as follows:

1. Pure-Culture Biological Methods

(a) Rot-Resistance Tests with *Chaetomium globosum*

(1) *Direct inoculation—sterilized specimen method.*—As this test can be used for either visual (qualitative) or breaking strength (quantitative) determinations, two types of test specimens were used. For visual examination, squares measuring 2 inches by 2 inches were used. Breaking strength specimens were cut 6 inches long in the warp direction by 1¼ inches in the filling direction. The specimens were raveled to an equal thread count which was approximately 1 inch in width in the filling direction before breaking strength was determined. As a control, untreated material, similar in all other respects to the treated material, was tested in the same manner as the test sample to verify the viability of the test organisms. A double-jacketed autoclave was used at a pressure of 15 pounds per square inch and an exhaust temperature of 121° C for steam sterilization. For leaching the specimens before the mildew tests, an apparatus was developed which consisted of a series of quart Mason jars so arranged that tap water was delivered to the bottom of each vessel at a rate of flow to permit five changes per hour. The water was maintained at 27° to 30° C and the pH was approximately neutral. Specimens were leached for 24 hours for most materials and 48 hours for treated leather. For incubation chambers, Petri dishes (10 cm) were used for visual tests. For quantitative tests, 16-ounce square bottles with modified screw caps were used. The modified screw caps were prepared by cutting circles 2 cm in diameter from the centers of the caps. Circular pieces of glass filter fabric (Owens-Corning Fiberglas—CS. 30A–20) were inserted on the inside of the caps.

The culture medium was made up as follows:

NH_4NO_3	3. 0 g.
K_2HPO_4	2. 0 g.
KH_2PO_4	2. 5 g.
$MgSO_4 \cdot 7H_2O$	2. 0 g.
Agar	20. 0 g.
Tap water	1. 0 liter.

The culture medium was melted in the autoclave, and 40 ml was poured into each bottle or sterile Petri dish. The Petri dishes were then left undisturbed until the agar had hardened. The bottles with culture medium were sterilized for 20 minutes, after which they were placed on their sides to harden.

In preparing the inoculum, scrapings from a ripe, fruiting culture of *Chaetomium globosum* USDA 1042.4, which completely covered a 10-cm Petri dish, were transferred to an Erlenmeyer flask containing 100 ml of sterile water by means of a sterile tungsten loop. The black spore clusters were squeezed against the sides of the flask with a sterile pipette until the tiny spores could be seen to go into suspension. Specimens that had been leached for 24 hours were sterilized for 1 hour in the autoclave. When the sterilized specimens were cool, one specimen was placed in contact with the agar medium in each bottle under aseptic conditions. One or two milliliters of inoculum, depending on the size of the specimen, was then distributed evenly over each specimen by means of a sterile pipette. The inoculated specimens were incubated for 7 days, for the qualitative test, and for 14 days, for the quantitative test at a temperature of 28° to 30° C and a relative humidity of 90 to 95 percent. In the quantitative test the breaking-strength specimens were removed from the bottles after 14 days of incubation, if not completely degraded, and were gently washed to remove any growth of mildew. The specimens were air-dried and then conditioned at 70° F and 65 percent relative humidity for 24 hours, after which they were broken. All averages of residual strength reported in this paper are based on 10 specimens for each test.

In the qualitative test the specimens were examined for growth of *Chaetomium globosum*. Any growth which could not be seen with the unaided eye was not considered to be significant. The average degree of growth was based on five specimens. The specimens were graded as follows:

No mildew.—No visible growth of mildew.

Very slight mildew.—The smallest amount of mildew growth which can be seen with the unaided eye—usually one or two isolated spots of growth. This is usually indicative of spotty treatment.

Slight mildew.—Small patches of growth, which

also indicate spotty treatment or perhaps differential leaching.

Moderate mildew.—Either many small patches of growth or a thin uniform growth over the entire specimen.

Heavy mildew.—Specimen is completely covered with mildew, but the underlying fabric structure is faintly visible.

Very heavy mildew.—Specimen is completely covered with a heavy growth of mildew, and the underlying fabric structure is no longer visible.

Aseptic conditions were maintained by carrying on all work in a dust-free room with filtered air. As a further precaution, the room was equipped with Sterilamps.

(2) *Mycelial mat method.*—Inasmuch as this test cannot be used for making qualitative visual observations because a specimen may show little or no visible growth on the upper surface and yet show a marked decrease in breaking strength, the test specimens were prepared for breaking-strength determinations as described in the previous test method (page 25). It was not necessary to use additional viability controls for this method, since each culture bottle contained its own control. Only culture bottles that had a well-developed 3-day mycelium were used. The apparatus, culture medium, and inoculum for this test were the same as described for the previous test (page 25), except that only 16-ounce square screwcap bottles were used. The culture medium was melted in the autoclave and was poured into 16-ounce square screwcap bottles with modified caps. The bottles containing agar were sterilized for 20 minutes, after which they were placed on their sides to harden. For the source of carbon, strips of filter paper measuring 1¾ by 5½ inches were sterilized in bottles for 1 hour. When cool, one strip of sterile filter paper was transferred to the surface of the hardened agar medium in each agar bottle under aseptic conditions. Two milliliters of inoculum were distributed evenly over each strip of filter paper in the agar bottles by means of a sterile pipette. The inoculated strips were then incubated for 3 days to develop mycelial mats. Bottles that failed to develop vigorous mats were discarded. At this point, unsterilized, leached test specimens were inoculated by immersing them in a fresh inoculum in a large beaker. Excess moisture was drained off by holding each specimen against the side of the beaker. The specimens were then laid out smoothly over the 3-day-old mycelial mats and were incubated at 28° to 30° C and a relative humidity of 90 to 95 percent for 14 days. The data were obtained as described in the preceding quantitative test method (page 25). Figure 8 shows a specimen of wood overgrown by *C. globosum* in the mycelium mat test.

(b) Mildew-Resistance Tests with *Aspergillus niger*

(1) *Mycelial mat method.*—Inasmuch as breaking strength had no significance in a test in which the test organisms were surface growers, the test specimens were cut 2 by 2 inches for visual observation. The apparatus for this test was the same as described for the preceding tests (page 25), except that only Petri dishes were used as incubation chambers. In order to accommodate liquid plasticizers, Petri dishes were modified by fitting them with Pyrex glass rings ½ inch in length by ½ inch in diameter. The rings were ground against the central surface of the Petri dish to assure a tight, leakproof fit. The hot sterile agar was poured around the rings, leaving a hollow cylinder in the center. The culture medium was the same as described for the preceding tests (page 25), except that 30 grams of sucrose per liter was added. The culture medium was sterilized in the autoclave for 20 minutes and was allowed to cool somewhat before pouring. About 40 ml of the hot sterile agar medium was poured into sterile Petri dishes under aseptic conditions. The plates were then covered and left undisturbed until the agar medium had hardened. The hardened agar medium was inoculated by first loading a sterile camel's-hair brush with spores from a Petri dish culture of *Aspergillus niger* USDA 215–4247 and then brushing the surface of the sterile agar medium uniformly under aseptic conditions. The inoculated Petri dishes were incubated for 42 to 48 hours at 28° to 30° C and 90- to 95-percent relative humidity until the white mycelium was evident over the entire surface of the agar medium. Cultures that did not show a vigorous growth of mycelium were discarded. No other viability controls were required. At this point leached test specimens were dipped in a fresh aqueous suspension of *A. niger* and were laid firmly on the mycelial mats and incubated at 28° to 30° C and 90- to 95-percent relative humidity for 14 days. At the end of the incubation period the test specimens were examined for growth of *A. niger*. Any growth which could not be seen with the unaided eye was not considered to be significant. The average degree of growth was based on five specimens. The specimens were graded as described in the qualitative procedure of the *Chaetomium*-direct inoculation method (page 25). Figure 9 shows the conclusion of a mycelial mat test with A. niger.

(2) *Sugar-free-medium method.*—The conditions of this test were similar to those of the *Aspergillus*—mycelial mat method, except that sucrose was not added to the medium. For the viability control, material that is known to support a growth of *A. niger* was tested in the same manner as the test sample to verify the viability of the test organisms. Test specimens were transferred to the sterile agar plates and were inoculated by dusting them with the spores of a Petri dish culture of *A. niger*. Dusting was effected with a dry, sterile camel's-hair brush. The inoculated specimens were incubated at 28° to 30° C and 90 to 95-percent

FIGURE 8. *Wood treated with inferior fungicide showing growth of Chaetomium globosum when tested by the mycelial mat method.*

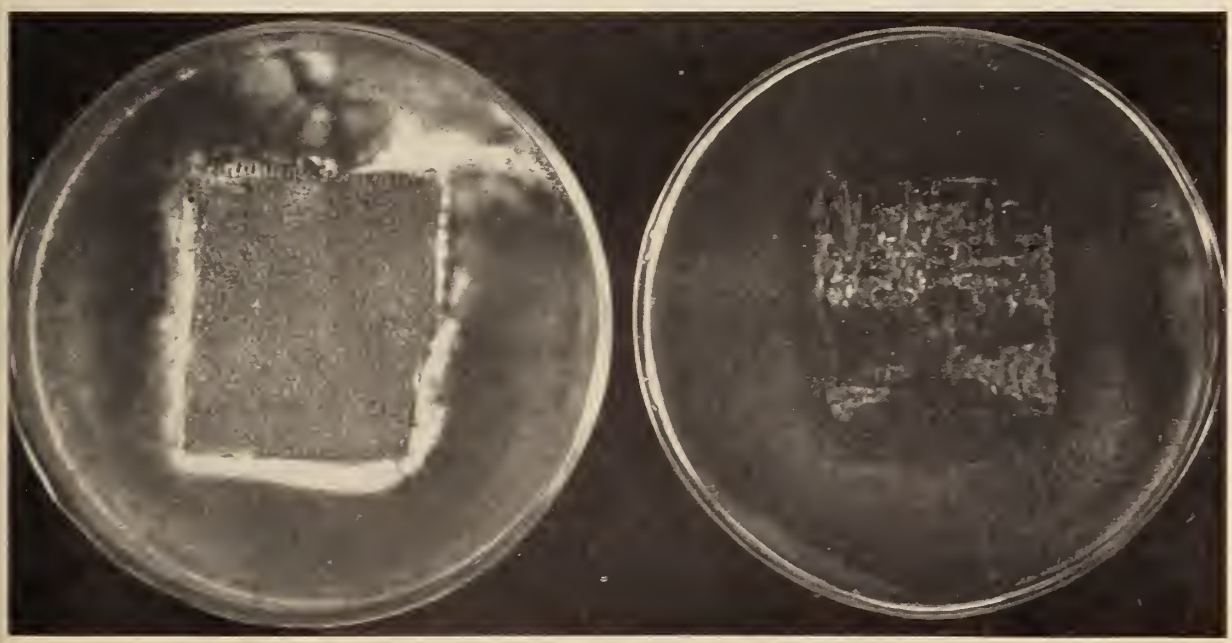

FIGURE 9. *Aspergillus niger test of mildew-resistant camouflage fabric.*

The specimen on the right had an insufficient concentration of the fungicide. The specimen on the left was adequately treated.

relative humidity for 14 days. The specimens were graded as described in the qualitative procedure of the *Chaetomium*-direct inoculation method (page 25).

2. Mixed-Culture Biological Test Methods

(a) Soil-Burial Test

The specimens for the soil-burial test were prepared as described for the quantitative procedures of the *Chaetomium* tests (page 25). For this test two types of control specimens were used. Untreated cotton duck was exposed in the soil beds throughout the periods of testing in order to verify the microbiological activity of the soil. The soil was considered to be in a satisfactory condition when the untreated controls deteriorated in from 7 to 10 days. The reference standard consisted of cotton fabric (similar in construction to the test fabric) treated with a solution of copper naphthenate of such concentration that 1.0-percent plus or minus 0.05-percent of copper was retained by the fabric. The fabric was impregnated with copper naphthenate with the aid of a household wringer. The desired roll setting was first determined by using Stoddard's solvent alone. Then strips of cloth 6 inches long in the warp direction and the full width of the material were immersed in copper naphthenate solution of the determined concentration until thoroughly wet. The strips were then passed through the rolls of the household wringer and were dried with constant motion to prevent migration of the fungicide. Before use the treated fabrics were analyzed for copper content. The soil used in this test was taken from the top 6 inches of a fertile topsoil known as Chester loam. The pH was 5.7, and the moisture equivalent was 22.6-percent, based on oven-dry weight. The clay content was 16.9-percent, and there was 2.0-percent of organic matter present. Stones and roots were removed by sieving before use. The soil was placed in wooden containers measuring 30 by 30 by 8 inches in height. The containers were kept in a tight, insulated cabinet in which the air temperature was maintained at 28° to 30° C, and the relative humidity was 95 to 97 percent. After moisture equilibrium had been established, it was rarely necessary to add water to the soil to maintain a 23-percent water content. Test strips were inserted vertically to a depth of 4 inches by means of a metal tool. Care was taken so that not more than ¼ inch of the lower end of the fabric was bent over to assist burial. Thirty strips of each test sample were buried so that 10 specimens could be removed after each of 3 periods of time. The burial periods were 3, 6, and 9 weeks. The specimens were removed from the soil beds at the end of each burial period, unless they were completely degraded, and were gently washed to remove soil particles. They were air-dried and then conditioned for 24 hours at 70° F and 65-percent relative humidity, after which they were broken.

(b) Soil-Suspension Test

Breaking-strength specimens were used as described in the quantitative *Chaetomium* tests (page 25). Untreated material similar in all other respects to the treated material was tested in the same manner as the test sample to verify the viability and cellulolytic activity of the test organisms. The apparatus and culture medium for this test was the same as described for *Chaetomium* tests, except that only 16-ounce screwcap bottles were used. The inoculum consisted of a thin suspension of composted soil containing 70 g of soil to 250 ml of distilled water. It was freshly prepared for each test from a stockpile of soil obtained from Miss Margaret Furry, Bureau of Human Nutrition and Home Economics, U. S. Department of Agriculture. Agar bottles were prepared as described in the *Chaetomium* tests (page 25). The test fabric was inoculated by immersing in the inoculum for 30 minutes with occasional agitation. Each strip was then removed from the mud and placed on the hardened agar surface in the culture bottle. The bottles were then incubated at 28° to 30° C and 90 to 95% relative humidity for 14 days. Data were obtained as described in the quantitative *Chaetomium* tests (page 25).

3. Accelerated Weathering Test

Breaking strength specimens (page 25), in a sufficient number for subsequent biological tests, were exposed in an artificial weathering machine for 360 hours. The apparatus consisted of a vertical carbon arc mounted at the center of a vertical metal cylinder. The arc was enclosed in a clear globe of No. 9200 Px Pyrex glass 0.0625 inch thick. The globe was cleaned whenever the carbons were changed, which was at least once in every 36 hours of operating time. The arc was operated at 17 amperes, with 140-volt 60-cycle alternating current. The test specimens were mounted on the inside of the cylinder facing the arc. The distance of the face of the specimen from the center of the arc was 14¾ inches. The cylinder rotated about the arc at a uniform speed of approximately three revolutions per hour. A water spray hit each specimen in turn for about 1 minute during each revolution of the cylinder. Ten specimens were always broken after a 360-hour exposure to determine whether there had been any deleterious photochemical effect. If there was no significant loss in strength, the remaining exposed specimens were then subjected to various mildew tests.

X. Experimental Results and Discussion

1. The results in table 1 indicate that cotton osnaburg is less resistant to *Chaetomium globosum* than jute burlap, although both are cellulosic materials and have approximately the same initial breaking strength. The reason for this difference may be found in the basic structure of the two fibers. As the cotton fiber has a lumen, the mycelial threads can penetrate inside, and deterioration proceeds from both inside and outside. Furthermore, it is possible that there is a physical rupture of the cell wall that would tend to hasten deterioration when it is measured by loss in breaking strength. On the other hand, the jute fiber may have a certain amount of pectin-like material associated with it, which may offer a slight initial resistance.

As experience has shown that every visible growth of *C. globosum* on cotton fabric is associated with a loss in strength corresponding to the degree of growth, it can readily be seen from table 1 that the 7-day results of 45 percent and 15 percent for burlap and osnaburg, respectively, are entirely reliable when reported on the basis of visible amount of growth in the qualitative test.

2. Table 1 shows the effect of fiber structure on the rate of growth of *C. globosum* and the resultant deterioration of the fibers, and table 2 shows how the thickness of fabrics, of presumably the same type of cotton fibers, affects the rate at which they are disintegrated in the soil. Logically, it might be reasoned that inasmuch as thicker fabrics offer a smaller relative surface per unit of fabric to the microorganism, it should take longer to degrade the larger quantity of fabric. This would appear to be true if one considered only the 4-ounce sheeting, the 8-ounce cotton osnaburg, and the 20-ounce cotton duck. These are all tightly woven fabrics and are quite comparable in this respect. However, the 37.5-ounce duck, which, according to its thickness, should be more resistant than the 20-ounce duck, is about half as resistant as the 20-ounce duck. Here the only difference is in tightness of weave. The 37.5-ounce duck is a very loosely woven fabric and would be more apt to absorb moisture than the more tightly woven fabrics. It may well be that the moisture holding capacity of cotton fabrics is of prime importance in their microbiological degradation.

TABLE 1. *Deterioration of untreated cellulosic fabrics by Chaetomium globosum*

Incubation period	Residual strength of—	
	Burlap	Osnaburg
Days	*Percent*	*Percent*
3	84	74
4	72	47
5	49	30
7	45	15
8	14	7
9	12	0
10	0	----------

TABLE 2. *Effect of fabric thickness on rate of deterioration in soil-burial test*

Burial period	Residual strength			
	Lightweight sheeting	Cotton osnaburg	20-oz cotton duck	37.5-oz cotton duck
Days	*Percent*	*Percent*	*Percent*	*Percent*
6	17	19	100	70
10	0	10	100	46
14	----------	0	97	38
21	----------	----------	27	15
28	----------	----------	7	0

3. Table 3 shows the results of vertical and of horizontal burial of cotton osnaburg strips. By the seventh day the vertical strips were completely deteriorated, whereas, by the ninth day, the horizontal strips retained 13 percent of their strength. Many workers concur in the opinion that most of the damage in soil burial occurs at the soil-air line. When a specimen is buried vertically to a depth of 4 inches, 2 inches of the specimen remain on the surface. Not only does the specimen have greater aeration at the soil-air line, but it also receives the attention of the different types of organisms that exist at different levels of the soil. According to Waksman, the top 4 inches of a soil are most active microbiologically. As to horizontal burial, the aeration of the specimens is especially poor. Restricting a specimen to one level of the soil may be restricting it to one type of organism. For these reasons it would appear to be advisable to employ vertical burial of specimens in the soil-burial test for maximum degradation.

TABLE 3. *Comparison of vertical and horizontal soil-burial tests*

Burial period	Residual strength	
	Vertical burial	Horizontal burial
Days	*Percent*	*Percent*
2	82	99
4	34	91
5	8	51
7	0	34
8	--	24
9	--	13

4. Table 4 shows the resistance to leaching of three copper compounds used for treating jute sandbags. In order to be able to recommend concentrations of these fungicides, which would permit a margin of safety, this rather rigorous test was carried out. The excellence of copper naphthenate stands out. After 96 hours of leach-

TABLE 4. *Effect of prolonged leaching at 70°C on mildew resistance of treated jute fabrics*

Treatment	Hours of extraction	Growth of *Chaetomium globosum*
Cuprammonium	24	None
	48	Do.
	72	Do.
	96	Do.
Copper ammonium fluoride	24	None.
	48	Do.
	72	Do.
	96	Moderate
Copper naphthenate	24	None
	48	Do.
	72	Do.
	96	Do.

ing at 70° C, the treated jute fabric is still resistant to *C. globosum*. Whereas 0.8 to 1.0 percent of copper naphthenate would be entirely satisfactory as a cellulosic fungicide, higher concentrations of cuprammonium and copper ammonium fluoride would be required to give the same protection.

5. Bartlett and Goll [15] investigated the possible photochemical oxidation of cellulose by copper compounds, acting as catalysts. Experiments with fabrics impregnated with 0.6 percent of copper naphthenate (based on metallic copper) gave no evidence of fabric tendering. On the other hand, copper sulfate in a similar concentration produced a great loss in tensile strength. They concluded that copper naphthenate does not hasten oxidative degeneration of cellulosic fabrics, although copper sulfate seems to have such an effect and might be the origin of reports about this phenomenon. Bayley and Weatherburn [20] exposed cotton, hemp, manila, and sisal cordage treated with 0.1 and 0.5 percent of copper as copper naphthenate for 7 summer months and found no increase in the rate of deterioration due to the presence of copper. In another paper [21] they reported that untreated No. 8 cotton duck showed substantial loss in breaking strength after outdoor exposure for 3 months. Similar fabric treated with 0.1 to 1.0 percent of copper naphthenate, copper hydroxy naphthenate, and copper oleate showed losses of breaking strength similar to that of the untreated fabric. However, when measured by the cuprammonium fluidity technique there was some evidence of increased actinic degradation.

In order to gather as much information as possible regarding the supposed photochemical effect of copper compounds, a wide range of concentrations of copper naphthenate was used in experiment 5.

In table 5, a, it can be seen that the untreated fabric is the only one that loses all its strength during the test. It would appear that the copper naphthenate has served as a protective coating rather than as an oxidative catalyst. With increase in copper concentration there is increased protection. Above 1.0 percent of copper con-

TABLE 5. *Effect of copper naphthenate on cotton osnaburg in presence of light*

(a) EXPOSURE TO ULTRAVIOLET LIGHT

Treatment of sample	Residual strength after—			Residual copper after—		
	50 hr	100 hr	150 hr	50 hr	100 hr	150 hr
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Untreated	31	10	0			
Copper naphthenate, 0.5%	38	11	5	0.31	0.17	0.04
Copper naphthenate, 1.0%	42	23	7	.74	.53	.41
Copper naphthenate, 2.0%	44	24	11	2.0	1.9	1.7
Copper naphthenate, 3.0%	55	37	19	3.0	2.9	2.8
Copper naphthenate, 4.0%	72	45	29	4.0	3.9	3.7

(b) EXPOSURE ON NBS ROOF

	Residual strength after—									
	1 mo	2 mo	3 mo	4 mo	5 mo	6 mo	7 mo	8 mo	9 mo	10 mo
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Untreated	93	64	62	51	49	47	46	38	34	33
Copper naphthenate, 0.5%	81	49	38	32	37	34	30	27	22	16
Copper naphthenate, 1.0%	84	48	34	28	29	25	21	20	17	15
Copper naphthenate, 2.0%	82	49	30	25	23	21	17	14	12	10
Copper naphthenate, 3.0%	82	50	36	32	30	26	24	20	19	16
Copper naphthenate, 4.0%	95	61	44	36	37	32	27	26	25	22
	Residual copper									
Copper naphthenate, 0.5%	0.4	0.1	0.06	0.02	0.008	0.005	0.004	0.003	0.003	0.003
Copper naphthenate, 1.0%	.8	.8	.4	.14	.01	.01	.01	.008	.008	.003
Copper naphthenate, 2.0%	1.9	1.9	1.5	1.2	.8	.7	.3	.3	.2	.098
Copper naphthenate, 3.0%	3.0	2.9	2.6	2.5	2.0	2.0	1.9	1.5	1.1	.96
Copper naphthenate, 4.0%	4.0	4.0	3.9	3.6	3.3	3.3	2.9	2.8	2.4	2.4

centration the loss in copper is negligible, whereas, at 0.5 and 1.0 percent the loss is considerable. However, if 0.3 percent of copper is considered to be the lower limit of fungicidal effectiveness, then even after 150 hours of exposure the 1.0-percent sample still retains 0.41 percent of copper.

The results of the roof exposures, listed in table 5, b, are not as clear-cut as are those for the ultraviolet-light-exposure samples. In the first month of exposure there is a loss in strength for all samples. However, the untreated fabric at 93 percent has not lost as much strength as the treated samples, up to the 3-percent concentration. It would seem that copper does have a deteriorative effect on cotton fabric. Yet, the 4.0-percent sample, which has more copper than any of the other samples, has a greater residual strength (95%) than the untreated fabric.

After 2 months the original fabric has dropped to 64-percent residual strength and the 4-percent copper fabric has dropped to 61 percent. The residual strength of the remaining copper-treated fabrics has dropped to 48 to 50 percent. After 3 months the untreated fabric has lost an additional 2 percent in strength, whereas the 4.0-percent-copper sample has dropped 17 percent, and now is not very much better than the other treated fabrics. It may be, assuming that there is a photochemical effect, that it takes longer for the ultraviolet rays to penetrate the 4-percent-copper sample and thus effect its degradation. In the ensuing months the untreated fabric continues to lose strength slowly until the tenth month, when only 33 percent of its original strength remains. The 4-percent sample also continues to lose strength slowly and has only 22 percent of strength retained at the end of 10 months. Of the remaining concentrations, the 2-percent-copper sample seems to show the greatest deterioration during each month after the second month. On the other hand, the 0.5-percent-concentration sample loses less strength as time goes on, and by the fifth month is as strong as the 4-percent sample. From there on until the ninth month it loses less strength than the 4-percent sample. Again, the copper loss is proportionately less for high concentrations than for low. The 0.5- and 1.0-percent concentrations would probably be ineffective as fungicides after the fourth month.

From the data in table 5, a, it appears that copper naphthenate does not increase the tendency to photochemical oxidation of cellulose under the conditions of the experiment. In fact, there appears to be a protective action with increasing concentration. However, table 5, b, indicates that although untreated fabric loses considerable strength during outdoor exposure, fabric treated with copper napthenate loses strength to a greater extent. Whether this increased loss is due to a photochemical effect in which the copper ion takes part is difficult to say. It would appear that the ultraviolet exposure, which completely deteriorated cotton osnaburg in 150 hours, was much more severe than the 10-month roof exposure. One possibility presents itself. The difference in deterioration may be due to differences in moisture. Although the samples were sprayed in the accelerated weathering test, the spray was of short duration, and, furthermore, copper naphthenate is known to render fabrics slightly water repellent. This combination might be sufficient to keep the treated samples relatively dry, whereas the untreated samples would tend to become wet enough to be affected photochemically. In this respect, the high degree of copper retention, above the 0.5-percent concentration, may be considered as circumstantial evidence pointing to the lack of leaching. On the other hand, the outdoor samples were subjected to alternate periods of sunshine and rain and morning dew. This is reflected in the relatively greater loss of copper. Furthermore, Bartlett and Goll [15] exposed their treated fabrics to ultraviolet light without the addition of moisture. In a 48-hour exposure test they found that copper naphthenate (0.6% Cu) treated cotton duck retained 75 percent of its strength, whereas the untreated fabric retained only 69 percent of its strength. The results of the 50-hour exposures in table 5, a, are approximately twice as severe but are in the same proportion.

6. Although most of the microbiological laboratories in the country have standardized on the use of *C. globosum*, several laboratories prefer other organisms or test methods with many claims for superiority. For example, Greathouse and Marsh have extolled the merits of *Myrothecium verrucaria* (*Metarrhizium glutinosum*), although they also find merit in *C. globosum* as well as a group of cellulolytic *Penicillia*. Furry is especially partial to the soil-suspension test, for which she makes great claims. Bertolet has discarded all pure-culture tests in favor of a soil-burial test with horizontal burial of specimens. Several laboratories favor the use of mixtures of organisms either in pure-culture technique or in mold chambers. Outstanding among these is the "Tropical Room" at the Engineer Board, Fort Belvoir, Va.

Table 6 shows that no single test can be used to determine the usefulness of a fungicide under all service conditions. Although the *Aspergillus niger* test (column a) is not normally used for testing fungicidal resistance of cellulosic materials, it was included in this series of tests to show the effect of *A. niger* on copper compounds. There is a slight growth of mildew on the 0.5-percent concentrations of copper napthenate and copper hydroxy naphthenate, but the higher concentrations of both compounds show no growth of mildew, although all specimens originally green

TABLE 6.—*Relative effectiveness of various mildew-proofing agents on 10-oz cotton duck as evaluated by a variety of test methods*

Fungicide		Growth of—		Residual strength							
		(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)		
Kind	Concentration	*Aspergillus niger*	*Chaetomium globosum*	*Chaetomium*-mycelial mat	Accelerated artificial weathering	Accelerated artificial weathering plus *Chaetomium*—mycelial mat	*Penicillium* 66	Soil suspension	Soil burial for—		
									3 wk	6 wk	9 wk
	Percent			*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
o-Phenylphenol	1.0	NM	NM	14	98	0	26	55	0	0	0
	2.0	NM	NM	18	92	0	39	79	0	0	0
	3.0	NM	NM	22	91	0	51	100	0	0	0
o-Phenylphenol, sodium salt	0.5	HM	MM	0	98	0	10	54	0	0	0
	1.0	SM	SM	9	96	0	11	63	0	0	0
	1.5	NM	NM	45	90	0	45	79	0	0	0
2, 4, 5-Trichlorophenol	1.0	NM	NM	16	93	0	70	66	97	0	0
	2.0	NM	NM	19	92	0	71	82	100	26	0
	3.0	NM	NM	26	90	0	90	94	100	61	0
2, 4, 5-Trichlorophenol, sodium salt	0.5	SM	NM	0	100	0	43	46	0	0	0
	1.0	NM	NM	9	100	0	47	51	0	0	0
	1.5	NM	NM	19	100	0	58	64	0	0	0
Chloro-2-phenylphenol	1.0	NM	NM	14	90	0	55	82	76	0	0
	2.0	NM	NM	36	83	0	64	82	77	0	0
	3.0	NM	NM	96	82	0	68	86	100	0	0
Chloro-2-phenylphenol, sodium salt	0.5	MM	NM	0	96	0	52	81	0	0	0
	1.0	SM	NM	11	98	0	66	85	0	0	0
	1.5	NM	NM	53	100	0	71	89	0	0	0
2-Chloro-4-phenylphenol	0.5	NM	NM	100	100	0	29	100	0	0	0
	1.0	NM	NM	100	93	0	80	100	0	0	0
	1.5	NM	NM	100	87	0	100	100	0	0	0
2-Chloro-4-phenylphenol, sodium salt	0.5	NM	NM	12	100	0	22	49	21	0	0
	1.0	NM	NM	33	100	0	35	78	33	0	0
	1.5	NM	NM	83	100	0	69	83	39	0	0
2-Bromo-4-phenylphenol	0.5	NM	NM	33	100	0	38	69	0	0	0
	1.0	NM	NM	65	97	0	67	73	0	0	0
	1.5	NM	NM	97	96	0	83	94	39	0	0
2-Bromo-4-phenylphenol, sodium salt	0.5	NM	NM	24	97	0	25	48	15	0	0
	1.0	NM	NM	52	100	0	29	57	57	40	0
	1.5	NM	NM	89	100	0	48	85	91	37	0
2, 3, 4, 6-Tetrachlorophenol	1.0	NM	NM	30	96	0	39	87	96	17	0
	2.0	NM	NM	36	94	6	45	98	100	44	0
	3.0	NM	NM	45	88	0	57	98	100	91	12
2, 3, 4, 6-Tetrachlorophenol, sodium salt	0.5	NM	NM	24	100	0	35	82	55	15	0
	1.0	NM	NM	29	100	0	36	91	89	40	0
	1.5	NM	NM	33	100	0	41	100	91	37	41
Pentachlorophenol	0.5	NM	NM	100	96	0	56	100	36	9	0
	1.0	NM	NM	100	85	0	100	100	83	46	38
	1.5	NM	NM	100	78	0	100	100	96	63	46
Pentachlorophenol, sodium salt	0.5	MM	NM	100	100	0	87	97	71	19	0
	1.0	SM	NM	100	100	0	100	100	88	47	0
	1.5	NM	NM	100	100	0	100	100	98	63	
2, 4, 6-Trichlorophenol	1.0	HM	HM	0	93	0	8	58	0	0	0
	2.0	HM	HM	0	90	0	15	64	0	0	0
	3.0	HM	HM	32	84	0	27	73	0	0	0
2,2′ Dihydroxy, 5,5′ Dichlorodiphenylmethane	0.5	NM	NM	23	79	0	53	76	100	55	10
	1.0	NM	NM	57	78	0	59	88	100	93	59
	1.5	NM	NM	76	72	0	61	100	100	100	97
Salicylanilide	0.5	NM	NM	48	94	0	23	72	86	22	0
	1.0	NM	NM	51	95	0	28	76	86	40	0
	1.5	NM	NM	69	95	0	30	85	87	37	0
Paranitrophenol	0.5	NM	HM	0	98	0	100	63	0	0	0
	1.0	NM	HM	0	94	0	100	74	28	0	0
	1.5	NM	HM	0	98	0	100	78	36	0	0
Phenyl mercuric acetate	0.5	NM	NM	58	98	0	50	79	21	0	0
	1.0	NM	NM	68	96	9	59	80	49	0	0
	1.5	NM	NM	90	100	24	63	89	97	0	0
Phenyl mercuric salicylate	0.5	NM	NM	48	100	0	67	69	8	0	0
	1.0	NM	NM	76	100	0	74	81	44	0	0
	1.5	NM	NM	99	100	23	92	96	76	0	0

TABLE 6. *Relative effectiveness of various mildew-proofing agents on 10-oz cotton duck as evaluated by a variety of test methods*—Continued

Fungicide		Growth of—		Residual strength							
		(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)		
Kind	Concentration	*Aspergillus niger*	*Chaetomium globosum*	*Chaetomium*-mycelial mat	Accelerated artificial weathering	Accelerated artificial weathering plus *Chaetomium*-mycelial mat	*Penicillium* 66	Soil suspension	Soil burial for—		
									3 wk	6 wk	9 wk
	Percent			*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Phenyl mercuric hydroxide	0.5	NM	NM	64	100	0	38	72	23	0	0
	1.0	NM	NM	66	100	0	53	82	62	0	0
	1.5	NM	NM	86	100	30	66	84	99	48	0
Phenyl mercuric oleate	0.5	NM	NM	45	100	10	52	63	42	0	0
	1.0	NM	NM	80	100	18	70	86	42	0	0
	1.5	NM	NM	98	100	30	89	97	78	0	0
Phenyl mercuric naphthenate	0.5	NM	NM	77	100	20	63	70	70	28	25
	1.0	NM	NM	82	94	43	70	72	75	66	45
	1.5	NM	NM	85	95	42	80	88	100	72	46
Phenyl mercuric acetoxy octadecanoic acid	1.0	NM	NM	0	100	0	76	86	77	0	0
	2.0	NM	NM	17	99	0	81	89	88	00	0
	3.0	NM	NM	22	99	0	83	94	90	0	0
Phenyl mercuric triethanolamine lactate	1.0	NM	NM	7	100	0	36	62	29	0	0
	2.0	NM	NM	9	97	0	53	67	34	0	0
	3.0	NM	NM	22	97	0	61	76	36	0	0
Pyridyl mercuric chloride	0.5	NM	NM	100	100	88	51	96	82	63	12
	1.0	NM	NM	100	100	97	59	96	100	73	43
	1.5	NM	NM	100	100	98	68	100	100	72	63
Pyridyl mercuric stearate	0.5	NM	NM	79	96	16	46	89	79	43	0
	1.0	NM	NM	95	98	67	50	99	91	89	38
	1.5	NM	NM	100	100	98	67	98	100	91	61
Pyridyl mercuric acetate	0.5	NM	NM	71	98	0	22	91	74	27	0
	1.0	NM	NM	89	100	0	35	89	89	41	0
	1.5	NM	NM	99	100	0	36	96	97	77	56
Mercaptobenzothiazole	0.5	NM	NM	27	93	0	32	55	40	0	0
	1.0	NM	NM	41	92	0	66	76	100	0	0
	1.5	NM	NM	50	89	0	93	98	100	72	0
2,3-Dichloronaphthoquinone	0.5	MM	NM	20	100	0	12	65	13	0	0
	1.0	MM	NM	62	97	0	22	65	41	0	0
	1.5	MM	NM	67	97	0	30	74	79	0	0
Zinc naphthenate	1.0	NM	NM	65	99	55	69	61	88	69	57
	1.5	NM	NM	67	100	57	84	79	91	74	60
	2.0	NM	NM	78	99	71	93	78	97	77	67
Zinc dimethyldithiocarbamate	0.5	NM	NM	63	100	64	54	74	98	81	69
	1.5	NM	NM	76	100	73	66	87	97	88	71
	2.0	NM	NM	89	99	86	72	94	100	98	80
Copper naphthenate	0.5	SM	NM	83	100	61	64	93	78	46	12
	1.0	NM	NM	89	100	89	85	92	94	83	61
	1.5	NM	NM	98	100	97	95	100	99	89	78
Copper hydroxynaphthenate	0.5	SM	NM	85	98	63	33	91	51	23	0
	1.0	NM	NM	89	98	58	66	94	70	54	14
	1.5	NM	NM	97	100	75	87	99	87	62	14
Copper oleate	0.5	HM	NM	76	100	33	45	79	13	0	0
	1.0	MM	NM	84	100	65	49	84	24	0	0
	1.5	MM	NM	91	100	83	53	98	45	0	0
Untreated		VHM	VHM	0	100	0	0	19	0	0	0

were decolorized, indicating a loss of copper. The presence of the naphthenic acid conferred a degree of mildew resistance upon all the specimens, including the 0.5-percent concentration. The copper oleate specimens, which were also decolorized, showed little resistance to *A. niger*. The loss of copper left only the oleic acid radical, which has no mildew-resistant power. Other compounds that showed little resistance to *A. niger* were 2,3-dichloronaphthoquinone, 2,4,6-trichlorophenol, lower concentrations of pentachlorophenol, and the sodium salts of chloro-2-phenylphenol, 2,4,5-trichlorophenol, and orthophenylphenol.

The *Chaetomium*-direct inoculation-sterilized specimen-qualitative test (column b) was first used in studying the mechanism of the microbiological deterioration of cotton. Later it was

used as a relatively rapid means for determining the thoroughness with which acceptable fungicides had been applied to cellulosic fabrics. With this method the National Bureau of Standards was able to test the fabric for 500 million sandbags during the first year of the recent war. This test was also useful in screening unknown fungicides. If a fabric, treated with an unknown compound, failed to pass this test, no further tests were necessary. In this investigation it is not surprising that *C. globosum* failed to grow on so many of the compounds. In the first place, the compounds were selected because of their excellence for some particular purpose, and, secondly, the concentrations are close to the upper limit of usefulness. Treatments that do not resist the growth of *C. globosum* in this test are: sodium salts of orthophenylphenol, 2,4,6-trichlorophenol, and paranitrophenol. Inasmuch as these compounds are rather unstable at high temperatures, it is quite likely that the use of steam sterilization is objectionable.

The *Chaetomium*-mycelial mat test represents the work of several laboratories. It was pioneered by Greathouse and Marsh and was further developed in the laboratories at the National Bureau of Standards. The results in column (c) show that very few compounds can resist the growth of *Chaetomium globosum* under the conditions of this test. A decided advantage of this test is that treated samples need not be subjected to steam sterilization—a condition not encountered in service. Treatments that resist *C. globosum* in this test are 3.0 percent of chloro-2-phenylphenol, 0.5 percent of 2-chloro-4-phenylphenol and its sodium salt, 1.5 percent of 2-bromo-4-phenyl-phenol and its sodium salt, 0.5 percent of pentachlorophenol and its sodium salt, 1.5 percent of phenyl mercuric acetate, 1.5 percent of phenyl mercuric salicylate, 1.5 percent of phenyl mercuric hydroxide, 1.0 percent of phenyl mercuric oleate, 1.0 percent of phenyl mercuric naphthenate, 0.5 percent of pyridyl mercuric chloride, 0.5 percent of pyridyl mercuric stearate, 1.0 percent of pyridyl mercuric acetate, 2 percent of zinc naphthenate, 1.5 percent of zinc dimethyl-dithio—carbamate, 0.5 percent of copper naphthenate, 0.5 percent of copper hydroxy naphthenate, and 0.5 percent of copper oleate. With the exception of the copper and mercury compounds, the concentrations of these compounds are somewhat higher than those customarily used.

Experience has shown that certain compounds promote deterioration of cellulose. Regardless of how they achieve their destructive ends, it is desirable to know whether a compound will affect a cellulosic fabric adversely even before it has the opportunity to protect the fabric from the ravages of the microorganisms. Such information could be obtained by lengthy outdoor exposures, but the accelerated weathering test is much faster. It must be emphasized that no claim is made as to the correlation of accelerated laboratory exposure with actual field exposure. There are many factors involved in outdoor exposure that are not yet fully understood. For example, copper naphthenate-treated fabrics actually show no loss of strength in the usual 360-hour exposure in the accelerated weathering test, whereas untreated fabrics do show a loss in strength. Yet the reverse is true in outdoor exposure, although here the untreated fabric also shows a loss in strength. At any rate, the accelerated weathering test has been useful in eliminating fungicides that break down in the presence of light and either liberate harmful products or are inactivated. In either case, the fabric is no longer protected.

Column (d) shows that the chlorinated phenols tend to weaken the fabric as the concentration is increased. This effect is serious only in the case of chloro-2-phenylphenol, pentachlorophenol, 2,4,-6-trichlorophenol and dihydroxydichlorodiphenylmethane. However, a breaking-strength test alone does not indicate whether any of these compounds has retained its fungicidal potency, even though the residual strength may be 100 percent.

In column (e) are listed the results of a *Chaetomium*-mycelial mat test, following 360 hours of accelerated weathering. The results are quite striking. Very few compounds are shown to possess the permanence necessary for the most rigorous service conditions. These superior compounds include pyridyl mercuric chloride, pyridyl mercuric stearate, zinc dimethyl dithiocarbamate, copper naphthenate, and copper oleate.

As a comparison of the effectiveness of *C. globosum* in the mat method another cellulolytic organism was chosen. *Penicillium* 66 was recommended by Dr. Paul Marsh, United States Department of Agriculture, Beltsville, Md. The results of tests with this organism are listed in column (f) Although it is a competent cellulolytic organism, it does not appear to be as tolerant of fungicides in general as *C. globosum*, although there are exceptions. Some of these are: 2-chloro-2-phenyl phenol and pentachlorophenol which are tolerated by *Penicillium* 66 at 0.5-percent concentration, whereas *C. globosum* is completely inhibited. Salicylanilide is better tolerated, to a slight extent, by *Penicillium* 66 than *C. globosum*. On the other hand, paranitrophenol completely inhibits *Penicillium* 66, whereas *C. globosum* is not affected by it at all. Phenyl mercuric acetoxy octadecanoic acid is vulnerable to *C. globosum*, although *Penicillium* 66 is fairly well inhibited by it. All pyridyl mercuric compounds are fairly easily attacked by *Penicillium* 66, although they are quite resistant to *C. globosum*.

It is obvious, then, that fungicides are effective only with respect to the test organisms used. Unfortunately, one cannot specify the type of or-

ganisms that shall attack a particular treated fabric in the field. Furthermore, it would be impossible to foresee all contingencies by attempting to use a large variety of test organisms. In this dilemma, the solution has been the soil-burial test, which is the most potent microbiological test in use at the present time. In column (h) are listed the results of the 3-, 6-, and 9-week exposures in the soil beds. The 6-week results show that great reliance may be placed on the *Chaetomium*-mycelial mat test, particularly after accelerated weathering. Two exceptions to this are pentachlorophenol and dihydroxydichlorodiphenylmethane, which are excellent in the soil-burial test.

In an effort to secure the obvious advantages of the many different types of organisms existing in composted soil, Furry developed the soil inoculum test. Unfortunately, the standardization of a test of this type entails the control of a great number of variables. For example, the soil used in this test was obtained from Miss Furry and had been stored for several months before use, with occasional watering to keep it from drying out. Strictly speaking, the soil should have been kept at standard conditions until needed for use in the test. However, it was not practical to do so. This lack of standardization in handling of the soil bank may account for the variation in test results encountered from laboratory to laboratory. The results of the soil-suspension test in this investigation appear in column (g).

Although this investigation was primarily concerned with the evaluation of the most widely used test methods, the use of such a large number and variety of fungicides provides an opportunity for evaluating these fungicides. For want of a better term, "fungicide," as used here, refers to all mildew-resistant compounds, regardless of their mode of action.

It is quite obvious that, in regard to any particular fungicide, resistance toward any particular test organism does not necessarily imply resistance to all organisms. Furthermore, a compound may be an effective fungicide under certain conditions but may lose its potency when subjected to the physical agents of weathering. Thus the chlorinated phenols, which are powerful fungicides, are limited in their use because of their high degree of solubility and because of their photochemical instability. With increasing concentration there is a tendency for these compounds to cause deterioration of the fabric during accelerated weathering. This effect is most pronounced in the case of pentachlorophenol, which shows a strength loss of 22 percent for the 1.5 percent concentration.

Dihydroxydichlorodiphenylmethane, although not a member of the Dowicide series of chlorinated phenols, is also a chlorinated phenol. Its photochemical effect on the fabric is even more severe than that of pctachlorophenol. At 1.5-percent concentration, 28 percent of the fabric strength was lost. However, unlike the other chlorinated phenols, this compound appears to be exceptionally resistant to soil burial, particularly at the 1.5-percent concentration.

Salicylanilide, which has been widely used under the trade name of Shirlan, is shown to be readily inactivated by accelerated weathering. Furthermore, its performance in the soil-burial test indicates either excessive solubility or the presence of organisms that hydrolyze the compound to salicylic acid and aniline.

Although paranitrophenol appears to be unusually resistant to *Penicillium* 66 at all concentrations, it is seen to be ineffective in all the other biological tests except the *A. niger* test. Although this compound appears to be of little value as a cotton-fabric fungicide, we shall see later (table 8) how it is effective as a leather fungicide.

The mercury derivatives have been demonstrated to be rather powerful fungicides. Although Penicillium 66 appears to tolerate these fungicides to a slightly greater degree than *C. globosum*, the difference does not seem to be significant. Furthermore, phenyl mercuric acetoxyoctadecanoic acid and phenyl mercuric triethanolamine lactate are tolerated to a greater extent by *C. globosum* than by *Penicillium* 66. On the other hand, the excellent pyridyl mercuric compounds show only moderate resistance to *Penicillium* 66, whereas *C. globosum* is almost completely inhibited by them. Pyridyl mercuric acetate appears to be the most soluble of the pyridyl mercuric group.

Mercaptobenzothiazole and 2,3-dichloronaphthoquinone are seen to be more or less ineffective against all organisms used. Inasmuch as these two compounds are known to be powerful fungicides, their ineffectiveness on fabrics is probably due to excessive solubility.

The two zinc compounds tested are seen to be moderately good fungicides. The protection, at higher concentrations, approaches the ideal of 90-percent residual strength. A tendency toward solubility is indicated by the results of the mildew test after accelerated weathering. These compounds, although not as effective as the copper compounds, can be recommended where the use of copper is objectionable, as, for instance, in rubber.

Of the three copper compounds tested, copper naphthenate is by far the superior fungicide. It is interesting to note that all of these compounds permitted a growth of *A. niger* to some extent. Where there was no growth, as in the higher concentrations of copper naphthenate and copper hydroxy naphthenate, the specimens were decolorized by the growth products in the mycelial mats. This phenomenon offers a clue as to the superiority of copper naphthenate and copper hydroxy naphthenate over copper oleate. The loss of color due to the action of the end products of *A. niger* may be simulated by immersing a test specimen in 0.4-percent solution of oxalic acid. Obviously,

the copper is removed. In the case of the naphthenate, the naphthenic acid that remains in the fabric has a degree of fungicidal potency and resists the growth of the microorganisms. However, the oleic acid residue of copper oleate is a well-recognized source of food for microorganisms.

7. Inasmuch as vegetable-tanned leather was found to support a vigorous growth of *A. niger* in the absence of the sucrose and minerals of a culture medium, it became desirable to determine the constituent of the leather that was promoting this growth. Table 7 shows that when degreased leather is subjected to the *A. niger* test, the growth is slight before leaching and very slight after leaching. This would indicate that the slight degree of growth is stimulated by the soluble materials in the leather, such as minerals and glucosides. When fatty oil is added to the leather, the growth both before and after leaching is heavy. When fatty oil and 1 percent of salicylanilide are added to the leather, there is no growth of *A. niger*, indicating that this fungicide at high concentration can inhibit the growth of *A. niger* when it obtains its nourishment from fatty oil. When leather grease was added to degreased leather, the growth of *A. niger* was very heavy both before and after leaching. Finally, when fatty oil, grease and 1 percent of salicylanilide were added to degreased leather, the salicylanilide was no longer able to completely inhibit the growth of *A. niger*. Apparently, the leather grease is the material that promotes the vigorous growth of *A. niger*. This corroborates previous work by Kanagy, et al. [90], in which it was shown that when vegetable-tanned leather was exposed to various microbiological tests the greatest damage to the leather was caused by stiffness due to a loss of grease.

TABLE 7. *Effect of constituents of vegetable-tanned leather on growth of Aspergillus niger*

Materials added to degreased vegetable-tanned leather	Growth of *Aspergillus niger*	
	Before leaching	Leached 48 hr
None	Slight	Very slight.
Fatty oil	Heavy	Heavy.
Leather grease	Very heavy	Very heavy.
Fatty oil plus grease plus Shirlan	Slight	Slight.
Fatty oil plus Shirlan	None	None.

8. As vegetable-tanned leathers are so susceptible to attack by molds, only the most powerful fungicides will give protection. However, what may be an effective fungicide for fabric may not be at all effective for leather. Furthermore, many fungicides cannot be used on leather because of their undesirable effects. For example, copper naphthenate cannot be used because it darkens leather. Mercury compounds cannot be used on account of their toxicity. Table 8 shows the results of the *A. niger* test on the more effective fungicides. An interesting compound is trimethyl cetyl ammonium pentachlorophenate (Hyamine 3258), which is said to be substantive to cellulose. However, it offers no protection to leather. Similarly, dihydroxydichlorodiphenylmethane is an excellent fabric fungicide, except in the presence of light, yet it has little effect as a leather preservative. Paranitrophenol and pentachlorophenol are of especial interest. Paranitrophenol was found to be effective at 0.35 percent and pentachlorophenol at 0.25 percent. However, both of these concentrations are too high from the point of view of toxicity to human beings. By combining these two fungicides in equal parts it is found that as low a combined concentration as 0.20 percent will effectively control the growth of *A. niger*. At this concentration the compound may be used for leather

TABLE 8.—*Relative effectiveness of various mildew-proofing agents on vegetable-tanned leather as evaluated by the Aspergillus niger test*

Fungicide	Concentration on leather	Growth of *Aspergillus niger*	
		Before leaching	Leached 48 hr.
	Percent		
8-Hydroxyquinoline	0.22	Slight	Moderate.
	.48	None	Slight.
	.72	do	None.
	1.06	do	Do.
Mereaptobenzothiazole	0.34	Moderate	Very heavy.
	.52	do	Heavy.
	.58	do	Do.
	1.07	do	Moderate.
Tetrabrom-ortho-cresol	0.21	do	Very heavy.
	.26	do	Do.
	.51	do	Heavy.
	.83	do	Moderate.
Beta-naphthol	.24	do	Heavy.
	.55	None	Moderate.
	.82	do	None.
	1.38	do	Do.
Trimethyl cetyl ammonium pentaehlorophenate.	0.29	Moderate	Heavy.
	.45	do	Do.
	.57	do	Moderate.
	.77	Slight	Slight.
Anilino methyl mereaptobenzothiazole	.28	Moderate	Very heavy.
	.45	Slight	Heavy.
	.76	do	Moderate.
	1.01	do	Do.
2, 2′ Dihydroxy-5, 5′ dichlorodiphenyl methane.	0.23	Moderate	Very heavy.
	.39	do	Heavy.
	.62	do	Do.
	1.03	do	Moderate.
Paranitrophenol	0.30	None	Do.
	.57	do	Do.
	.65	do	None.
	1.01	do	Do.
Pentaehlorophenol	0.25	do	Slight.
	.59	do	None.
	.89	do	Do.
	1.16	do	Do.
Paranitrophenol and pentaehlorophenol.	0.11	Slight	Moderate.
	.20	None	None.
	.26	do	Do.
	.37	do	Do.
Salicylanilide	.26	Very heavy	Very heavy.
	.41	Heavy	Do.
	.73	do	Do.
	1.24	Moderate	Do.

articles that come in contact with the human body. This fungicidal combination, developed at the National Bureau of Standards, is the basis of a mixture for use on leathers and was supplied to the soldiers in the tropical regions during the latter part of the recent war. It was found to be highly satisfactory.

9. Table 9, a, shows the susceptibility of 15 unplasticized polymers to *Aspergillus niger*. The results of the test with the complete medium (mycelial mat method) indicate that only urea-formaldehyde has any fungicidal power. The remaining plastics all permit a growth of *A. niger* over them, varying from slight to heavy. This test alone would not suffice to tell whether these materials actually supported a growth of the organism. To be sure, a material, such as urea-formaldehyde, which gives off formaldehyde, manifests its fungicidal character in this test. The results of the tests with sugar-free medium, however, show that only cellulose nitrate, polyvinyl acetate, phenol-formaldehyde, and melamine-formaldehyde are sources of food for *A. niger*. Although none of the remaining polymers supports a growth of mildew, it does not prevent the growth of mildew. These tests indicate that when these materials are accompanied by substances that promote the growth of micro-organisms, no inhibition will take place because of the polymeric material itself, except with urea—formaldehyde. Presumably when commercial plastic materials support a growth of mildew, components other than the pure polymer are responsible for it.

Table 9, b, which lists the susceptibility of 83 plasticizers to *A. niger*, shows the prime source of mold susceptibility of plastic materials. Although many of the plasticizers show no growth of mildew in the sugar-free test, indicating that they are not sources of mold nutrition, all of them show some degree of permissive growth when sugar is added to the medium. Of the plasticizers that support a growth of mildew on sugar-free agar, the worst offenders include: glycol sebacate resin, sebacic acid alkyl resins, castor oil and ricinoleate derivatives, tung oil, glyceryl triacetate, and pentaerythritol triacetate monopropionate. It is interesting to note that of the 10 pentaerythritol derivatives tested, three showed no growth, five showed slight growth, one showed moderate growth, and one showed heavy growth. In like manner it is difficult to correlate the results of the remaining compounds on the basis of chemical structure, particularly since, in general, there is less similarity in structure among the other compounds than among the pentaerythritols. However, certain trends are evident. As a group the phthalates may be said to offer no source of nutriment for the development of *A. niger* with several exceptions. Ricinoleates invariably support copious growths of this organism as do the sebacates and stearates.

TABLE 9. *Mold susceptibility of pure plastics and plasticizers*

a. PURE PLASTICS

Pure polymer	Growth of *Aspergillus niger*	
	Complete medium	Sugar-free medium
Cellulose acetate	Slight	None.
Cellulose acetate butyrate	do	Do.
Cellulose acetate propionate	Moderate	Do.
Cellulose nitrate	do	Moderate.
Ethyl cellulose	do	None.
Polyethylene	Slight	Do.
Polymethylmethacrylate	do	Do.
Polystyrene	Moderate	Do.
Polyvinyl acetate	Heavy	Moderate.
Polyvinyl chloride	Moderate	None.
Polyvinyl acetate-chloride VYNW	Slight	Do.
Polyvinyl acetate-chloride VYHH	Moderate	Do.
Phenol-formaldehyde	do	Slight.
Melamine-formaldehyde	Heavy	Moderate.
Urea-formaldehyde	None	None.

b. PLASTICIZERS

Hydrogenated methyl abietate	Moderate	None.
Tri-n-butyl aconitate	do	Slight.
Triethyl aconitate	do	Do.
Ethyl-o-benzoyl benzoate	do	None.
Benzyl benzoate	Heavy	Moderate.
Tri-n-butyl citrate	Moderate	None.
Triethyl citrate	Heavy	Do.
Glyceryl triacetate	do	Heavy.
Diethylene glycol ethyl ether acetate	Moderate	Moderate.
Diacetate of 2-nitro-2-methyl-1,3-propanediol	do	None.
Dipropionate of 2-nitro-2-methyl-1,3-propanediol.	Heavy	Do.
Dicthylene glycol dipropionate	Moderate	Do.
Triethylene glycol di-(2-ethylhexoate)	Heavy	Do.
Triethylene glycol di-(2-ethylbutyrate)	do	Do.
Polyethylene glycol di-(2-ethylhexoate)	do	Do.
Ethyl phthalyl ethyl glycolate	Slight	Slight.
Methyl phthalyl ethyl glycolate	Moderate	None.
Butyl phthalyl butyl glycolate	do	Moderate.
Ethylene glycol methyl ether oleate	do	Do.
Dipentaerythritol hexaacetate	Heavy	None.
Dipentaerythritol hexapropionate	Moderate	Do.
Dipentaerythritol hexabutyrate	do	Do.
Pentaerythritol diacetate-dibutyrate	do	Slight.
Pentaerythritol diacetate-dipropionate	do	Do.
Pentaerythritol monoacetate-tripropionate	Heavy	Do.
Pentaerythritol triacetate monopropionate	do	Heavy.
Peutaerythritol tripropionate monomyristate	do	Moderate
Pentaerythritol tetrabutyrate	Moderate	Slight.
Pentaerythritol tetrapropionate	Heavy	Do.
Triethyl phosphate	Moderate	None.
Tributyl phosphate	Slight	Do.
Triphenyl phosphate	Moderate	Slight.
Tricresyl phosphate	do	None.
Tri-(2-nitro-2-methylpropyl) phosphate	do	Moderate.
Monophenyl *di*-(*p*-tert.-butylphenyl) phosphate	do	Slight.
Diphenyl mono-*o*-xenyl phosphate	Heavy	Do.
Di-o-xenyl monophenyl phosphate	Moderate	None.
Tri-(*p*-tert. butylphenyl) phosphate	Heavy	Do.
Tri-(*o*-xenyl) phosphate	Moderate	Do.
Dimethyl phthalate	do	Do.
Diethyl phthalate	do	Do.
Di-n-propyl phthalate	Heavy	Do.
Di-isopropyl phthalate	Moderate	Do.
Dibutyl phthalate	do	Do.
Di-isobutyl phthalate	Slight	Do.
Diamyl phthalate	Moderate	Do.
Dicapryl phthalate	do	Slight.
Dioctyl phthalate	do	None.
Di-(2-ethylhexyl) phthalate	Heavy	Do.
Dieyclohexyl phthalate	do	Do.

TABLE 9. *Mold susceptibility of pure plastics and plasticizers*—Continued

h. PLASTICIZERS—Continued

	Growth of *Aspergillus niger*	
	Complete medium	Sugar-free medium
Dibenzyl phthalate	Moderate	Do.
Dimethoxy ethyl phthalate	do	Do.
Diethoxy ethyl phthalate	Slight	Do.
Dibutoxy ethyl phthalate	Moderate	Do.
Methyl-2-methyl-2-nitropropyl phthalate	Heavy	Do.
Ethyl-2-methyl-2-nitropropyl phthalate	do	Slight.
Butyl-2-methyl-2-nitropropyl phthalate	Moderate	None.
Bis-(diethylene glycol ethyl ether) phthalate	do	Do.
Glycol sebacate resin	Very heavy	Very heavy.
Sebacic acid alkyd resin-G25	do	Do.
Sebacic acid alkyd resin-RG-2	do	Do.
Sebacic acid alkyd resin RG-20	do	Do.
Butyl acetyl ricinoleate	do	Do.
Ethylene glycol methyl ether acetyl ricinoleate	do	Heavy.
Dimethyl sebacate	Moderate	Moderate.
Dihutyl sebacate	Heavy	Do.
n-Butyl stearate	do	Do.
Cyclohexyl stearate	do	Do.
Butoxy ethyl stearate	Moderate	Do.
Di-n-hutyl tartrate	do	None.
Ethyl-*p*-toluenesulfonate	do	Do.
o-Cresyl-*p*-toluenesulfonate	Slight	Do.
o- and *p*-Toluene ethylsulfonamide	Moderate	Do.
Triethyl tricarhallylate	Slight	Slight.
Tri-*n*-butyl tricarhallylate	Heavy	Do.
Castor oil	Very heavy	Heavy.
Tung oil	Heavy	Do.
Diphenyl	Slight	None.
Diamylnaphthalene	do	Do.
Cyclohexyl lactate	Moderate	Do.
Methylcyclohexyl oxalate	Slight	Do.
Diphenylsulfone	Moderate	Do.
Triphenylguanidine	Slight	Do.

By making use of the information in table 9, a and b, it has been possible to compound a plastic material that does not support a growth of mildew. For example, polyvinyl acetate-chloride copolymer has been successfully plasticized with tricresyl phosphate as a result of this investigation. Previously, the most common plasticizers had been the ricinoleates.

10. As a result of the investigation of the mold susceptibility of plastics and plasticizers, other materials that had formerly been considered to be inert, as far as mold nutrition was concerned, were investigated in the same manner. The results are recorded in table 10. It is not at all surprising to find that natural crepe rubber supports mold growth. The literature on mildewed crepe rubber is extensive. However, of the synthetic rubbers both Buna S and butyl rubber support a growth of *A. niger*. Neoprene, on the other hand, does not support a growth of mildew, although it does not inhibit growth when sugar is added to the culture medium.

TABLE 10. *Mold susceptibility of various materials*

Material	Growth of *Aspergillus Niger*	
	Complete medium	Sugar-free medium
Natural crepe rubber	Very heavy	Heavy.
Buna S	Heavy	Moderate.
Butyl (isoprene)	do	Do.
Neoprene	do	None.
Nylon fabric	do	Do.
Glass fabric	do	Do.
Mineral wool	None	Do.

Nylon fabric, glass fabric, and mineral wool all offer no nourishment to *A. niger*. However, mineral wool is the only one that resists the growth of mildew even when sugar is added.

As a result of this investigation it became possible to recommend a material for use in fabricating mildew-resistant portable water tanks. Canvas tanks had formerly been used, but because of their susceptibility to rot it became necessary to develop mildew-resistant treatments for them. Unfortunately, none of the superior fungicides could be used for this purpose because of the possible contamination of drinking water. Consequently, the value of a material such as neoprene-coated nylon can be readily seen.

XI. Summary and Conclusions

A series of 10 investigations is reported in which the basic problems of the testing of mildew-resistant treatments for organic materials are covered.

1. It has been shown that cellulosic fibers of different origins have varying resistance to microorganisms. Cotton fibers are more easily deteriorated than jute fibers.

2. Fabrics of the same cellulosic fibers, which differ as to thickness and tightness of weave, also vary in their resistance to microbiological attack. In general, thicker fabrics tend to resist deterioration for longer periods, unless they are loosely woven.

3. It has been shown that the results in the soil-burial test can be affected by the manner in which specimens are buried. Vertically buried specimens appear to offer greater opportunities for microbiological attack than horizontally buried specimens.

4. The excellence of copper naphthenate as compared to two other copper compounds is demonstrated by its resistance to leaching for 96-hour at 70° C.

5. Copper naphthenate appears to protect cellulosic fabrics from photochemical degradation to a slight extent when exposed to strong ultraviolet rays, but the situation is reversed when the

same samples are exposed outdoors for as long as 10 months. It appears that in the presence of moisture and light, as in the outdoor exposure, there is a slight tendency for copper naphthenate to reduce the breaking strength of cellulosic fabrics. In the absence of large quantities of moisture, as in the ultraviolet exposure, copper naphthenate tends to retard the degradation of cellulosic fabrics.

6. For the purpose of evaluating cotton fabrics treated with mildew-resistant compounds, eight test methods have been studied. No single test offers a complete picture of the performance of a particular treatment under all service conditions. The tests that stand out as most useful are *Chaetomium*-Mycelial mat test; Accelerated weathering test, followed by a subsequent *Chaetomium* test; and the soil-burial test. Where the particular conditions of service are known, it may be sufficient to select a single test. But where a mildew-resistant treatment is required to protect under a wide variety of conditions, all three of the above tests should be used.

In evaluating these test methods, 35 of the most commonly used fungicides were used. The superiority of the copper compounds, especially copper naphthenate, has been demonstrated. Although not as effective, zinc naphthenate and zinc dimethyldithiocarbamate have been shown to be acceptable substitutes where copper compounds cannot be used. The chlorinated phenols have been shown to be excessively soluble and rather photochemically unstable. However, in the absence of light, pentachlorophenol and dihydroxydichlorodiphenylmethane would be excellent fabric fungicides. The pyridyl mercuric compounds appear to be the best of the mercury fungicides although the acetate is probably too soluble for general use. Finally, it is obvious that no single compound may be considered the universal fungicide. Even copper naphthenate, which withstands extemely rigorous exposure, is not the ideal treatment. However, with a knowledge of the end use of the treated fabric, it is possible to select an effective fungicide from these 35 compounds.

7. The material that imparts to vegetable-tanned leather its great susceptibility to mold growth appears to be the grease commonly used in leather manufacture.

8. Paranitrophenol and pentachlorophenol have been demonstrated to be effective fungicides for vegetable-tanned leather. Inasmuch as the effective concentrations of these compounds are too high, for practical reasons, a compound containing equal parts of these two fungicides has been developed in which the combination is effective at as low a concentration as 0.20 percent.

9. Fifteen unplasticized polymers and 83 plasticizers have been investigated for resistance to *Aspergillus niger*. Of all the materials tested only ureaformaldehyde showed a resistance to the growth of *A. niger* on a complete medium. On sugar-free medium, 4 polymers and 37 plasticizers were shown to support a growth of *A. niger*. The relatively large proportion of mold-susceptible plasticizers indicates the need for careful selection of plasticizers in compounding a plastic material. This data can be used in the selection of resistant materials for various plastic formulations.

10. A group of seven miscellaneous materials has been investigated for resistance to *A. niger*. Of all the materials tested only mineral wool resisted the growth of *A. niger* on a complete medium. On sugar-free medium, natural crepe rubber, Buna S and butyl rubber manifested the ability to support a growth of *A. niger*. Neoprene, nylon fabric, glass fabric, and mineral wool failed to support the growth of the organism.

XII. References

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